

Cytogenetic and molecular aberration data for oncogenetic data analysis projects. *M. Baudis*. Pediatrics, University of Florida, Gainesville, FL.

Main targets in the analysis of cytogenetic abnormalities in human malignancies are 1. identification of "hot spots" as presumptive loci of genes involved in neoplastic transformation and growth, and 2. identification of genomic markers aiding in differential diagnosis and prognostic prediction. Whereas banding analysis of tumor metaphase spreads has led to groundbreaking insights in oncogenetic mechanisms, metaphase and interphase based DNA hybridization techniques (FISH, CGH, mFISH, SKY) have increasingly been applied over the last decade. Currently, genomic array technologies gain in popularity due to the combination of high spatial resolution, independence from fresh tumor tissue, possible complete genome coverage and supposedly high automatization potential. For combining different types of genomic screening data, experimental readouts have to be reduced to information atoms, representing the status of a given interval (chromosomal band or genomic map). For the Progenetix database project (www.progenetix.net), evaluation software for ISCN 1995 conform karyotype and CGH annotations has been developed. As example for the integration of available data sources, 715 breast adenocarcinomas from the Mitelman database and 350 cases analyzed by CGH from Progenetix were compared using two-sided Kolmogorov-Smirnov test and correlation analysis, showing unequivocal similarity in the chromosomal deletion patterns.

Initially developed as a collection of data from published CGH projects, the Progenetix database has become a general repository for supervised genomic aberration data. Besides chromosomal band based annotations, the current evolution of array based CGH variants from proof of concept status to reference screening methods has warranted the development of a general data exchange format based on genomic intervals. Here, the draft version of an XML based data exchange format will be presented. A unified format for genomic aberration data will support data mining efforts aimed at the identification of genomic hot spots and disease specific aberration patterns, and simplify the integration with results from expression array experiments for the identification of relevant target genes.

Small upstream deletions of *ABL1* are rare in Chronic Myelogenous Leukemia. *A.M. Marion*¹, *P.K. Rogan*¹, *W. Flejter*², *D. Persons*³, *J. Cowan*⁴, *J.H.M. Knoll*¹. 1) Children's Mercy Hospital, University of Missouri-Kansas City; 2) Esoterix Oncology, Brentwood, TN; 3) Kansas University Medical Center, Kansas City; 4) Tufts-New England Medical Center, Boston, MA.

Many chronic myelogenous leukemia (CML) patients with poor prognoses have large chromosome 9 deletions upstream of *ABL1* that extend at least 450 kb and encompass at least 4 genes (*ASS*, *FBP3*, *PRDM12*, *RRP4*). We hypothesized that some other CML patients might exhibit smaller deletions which are not detectable with the commercial FISH probe that spans all of these loci. A dense array of short single copy (sc) FISH probes (1272 - 3965 bp in length) was developed directly from the genome reference sequence for each of these genes. We validated 15 sc probes in the interval upstream of *ABL1*, an average density of 1 probe per 28 kb; and 15 probes within *ABL1*, a density of 1 per 14 kb. Probe subsets were hybridized to diagnostic cytogenetic preparations from 71 unselected CML patients harboring t[9;22]. Six patients with large deletions were detected. FISH with sc probes from 5' *ABL1* (a 2207 bp probe that includes exon 1 of *ABL1* and 119 bp of IVS1b; and 1552 bp and 1272 bp probes derived from the middle of IVS1b) did not reveal smaller deletions in the remaining samples. We also excluded noncontiguous deletions with a 3005 bp probe from *FBP3*. Thus, we cannot attribute adverse outcomes in deletion patients to hemizyosity of particular genes in this interval. This study also localized various translocation breakage intervals within IVS1b (position 124,604,542-124,725,532; <http://genome.ucsc.edu: hg12>) of *ABL1* in non-deletion patients. Forty-five patients had a translocation breakpoint distal of coordinate 124,685,740; at least 6 patients distal of 124,604,661; 12 patients within a 26 kb region (124,604,661-124,630,536); and single individuals between 124,632,735-124,670,404 and 124,592,531-124,602,455. From these data, there appears to be preference for breakage in the COOH-terminal half of IVS1B, a finding that contrasts with published data. The *ABL1* breakage interval distributions in patients with der(9) deletions were similar to those seen in non-deletion samples, consistent with common initiation sites.

Are Meningiomas Benign Tumors: A Cytogenetics Approach. *C. Isaza¹, M.I. Escobar¹, A. Montoya², J. Sanchez³.*

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Objective: To demonstrate that in vitro culture of meningiomas is useful to determine cellular behavior, growth rate and karyotype; this data added to histopathology may be used to better determine prognosis and recurrence. **Materials and Methods:** Cells from 54 tumor samples taken from surgery of meningiomas in any location of the brain were cultured in vitro. The cellular growth rate was determined and karyotypes were prepared to assay for chromosomal alterations. **Results:** 52% of the meningiomas cultured successfully were not benign and were in progression having a rate of recurrence higher than 10%. **Conclusions:** Study of the cytogenetics and in vitro behavior of tumor cells may be an excellent complement to the surgical result and the pathological study, helping establish the risk of recurrence and the prognosis of the patient. **Financial Support:** Colciencias and Universidad del Valle.

Identification of genetic alterations in pediatric ependymal and embryonal central nervous system tumors by metaphase and arrays-based CGH. *M. Yoshimoto*¹, *N.S. Silva*², *J. Bayani*¹, *P.A.S. Nuin*³, *S. Cavalleiro*⁴, *J.A.D. Andrade*⁵, *M. Zielenska*⁶, *J.A. Squire*¹, *S.R.C. Toledo*^{2,5}. 1) Ontario Cancer Institute, Toronto, Ontario, Canada; 2) Instituto de Oncologia Pediátrica, São Paulo, Brazil; 3) Department of Biology, McMaster University, Hamilton, Ontario, Canada; 4) Divison of Neurosurgery, Universidade Federal de São Paulo, Brazil; 5) Division of Genetics, Universidade Federal de São Paulo, Brazil; 6) Department of Pediatric Laboratory Medicine, Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada.

Genetic investigations, primarily in medulloblastomas, have contributed significantly to our overall understanding of embryonal tumor development of central nervous system (CNS). In contrast, there is little information concerning the genetic changes in ependymomas. The main purpose of this study was to characterize genomic imbalances in pediatric embryonal and ependymal tumors by conventional metaphase-based comparative genomic hybridization (CGH). In all ependymomas CGH copy number profiles indicated that no imbalance was present. The finding of normal profiles suggests that the development of ependymomas at younger age is independent of the acquisition of copy number imbalance per se. In contrast, observed abnormal CGH profiles including gains of 16p and amplification of 5p in medulloblastomas from patients presenting with a progressive and fatal disease course. The amplified genomic region mapped to cytoband 5p15.33 with concurrent non-syntenic amplification at cytoband 2p24. This study illustrates the value of understanding the genomic mechanisms underlying pediatric CNS tumors drawn from Brazilian populations to identify novel biological markers to provide a molecular basis for future prognostic assessment. (Research supported by CAPES, Projeto Crianca e Vida and GRAACC).

Dual color break apart FISH probes: applications and limitations. *S. Adhvaryu*. Dept Pathology, Univ Texas Health Sci Ctr, San Antonio, TX.

Due to their ability to pick up variant translocations involving important loci involved in leukemia-lymphoma, the interest in use of dual color split-apart FISH probes has increased. We encountered two cases of AML that highlight limitation of using these probes on interphase nuclei. This point will be emphasized with help of following two cases:(I) A bone marrow, with a history of possible Acute Myeloid Leukemia was provisionally diagnosed as del(16)(q22). The FISH probe LSI CBF β Dual Color, Break Apart Rearrangement was used. The interphase nuclei showed normal pattern of two fusion (2F) signals. The metaphases showed one fusion signal on one chromosome 16 and the other F-signal on 3q. The abnormality was identified as 46,XX,t(3;16)(q28;q21). (II) A peripheral blood specimen was received with a diagnosis of possible Acute Pro-myelocytic Leukemia. The analysis showed a t(3;17) and a der(15), with no evidence for the typical t(15;17). FISH analysis using LSI RARA Dual Color, Break Apart Rearrangement probe was used. The analysis showed fusion signal on one 17q, whereas, the other chromosome 17 showed red and green signals separated from each other. The interphase nuclei showed abnormal pattern of one orange, one green and one fusion (1O1G1F) signals. Use of only interphase FISH could have missed detection of exact rearrangement in both the cases. Details of both the cases, including the clinical outcomes will be presented emphasizing the limitation of interphase FISH as a stand alone technique.

Genetic Instability on Benign Prostatic Hyperplasia(BPH). A. Amiel¹, I. Leibowitch^{2,3}, T. Sivan^{1,3}, M.D. Fejgin^{1,3}. 1) Genetic Institute, Meir Hospital, Kfar-Saba, Israel; 2) Department of Urology; 3) Tel Aviv University Medical School.

Genetic instability parameters like aneuploidy and replication pattern are known to be associated with pre-malignant and malignant conditions. Aneuploidy is known to be involved in prostate cancer and less in BPH. In order to assess replication and aneuploidy we applied FISH technique to BPH tissues taken from 34 men aged 51-91 (mean 72). For gene replication assay the RB-1 and 21q22 loci were used. Monosomy of chromosome Y (which has already been reported in BPH) was found in 35% of the samples. Other abnormalities found were monosomy of chromosome 7 (in 26% of the samples) and excess of chromosome X (in 6% of the samples). These last two abnormalities have been reported in prostate cancer but not in BPH. As to the gene replication assay, in both loci there was a shift from synchronized bi-allelic replication to asynchronized monoallelic replication compared to control cells ($p < 0.001$), as previously described in pre-malignant and malignant conditions. The two loci replicate earlier in the BPH tissue compared to control cells ($p < 0.05$). To conclude- Molecular cytogenetic parameters like aneuploidy and replication pattern which are known to reflect chromosomal instability, may be part of the tumor genesis process. It is possible that in BPH, the presence of genetic instability may represent the proliferative hyperplastic process and not necessarily a malignant potential.

An isolated tetrasomy 11 in a case of acute myeloid leukemia. *R. Ben Abdelali¹, H. Sennana¹, S. Ayari², H. El Ghezal¹, A. Hafsia², A. Saad¹.* 1) Laboratory of Cytogenetics, Farhat Hached Hospital, Sousse, Tunisia; 2) Hematology Department Aziza Othmana Hospital, Tunis, Tunisia.

Tetrasomy 11 as a sole chromosomal abnormality is very rare in AML and its clinical and prognosis significance remain largely unknown. In the current study we present clinical and laboratory characteristics and outcome data of de novo AML-M5 patient with tetrasomy 11 at diagnosis. Case report: A 43 year old woman was admitted because of fever and asthenia associated with cervical lymphadenopathies. Physical examination on admission revealed a pallor, signs of acute bronchitis and cervical lymphadenopathies. No hepato- or splenomegaly was noticed. Her peripheral blood count showed white blood cells 70,000/mL; hemoglobin 6.9 g/dL; and a platelet count of 110,000/mL. All of the bone marrow cells were myeloblasts of M5 morphology (FAB). At diagnosis, conventional cytogenetic analysis showed: 48,XX, +11, +11[9] / 46,XX[13]. FISH study using LSI MLL (11q23) dual color DNA probe revealed four separate fused signals in most of interphase cells, but not in any metaphase cells. The patient was treated with chemotherapy (aracytine and idarubicin). She did not respond to chemotherapy and died of sepsis occurred with pseudomonas perineal cellulite 13 days after first presentation. In a review of the literature 2 cases of tetrasomy 11 as sole cytogenetic abnormality have been reported. One of them was diagnosed with chronic myelomonocytic leukemia and the other with acute myelomonocytic leukemia. For both there is no description of clinical and hematological features. More cases are recommended for making the assessment of molecular and clinical characteristics of this chromosomal abnormality.

Co-amplification of 12q14-q21.3 and 5p14-pter in a Liposarcoma with atypical cytogenetic results. *M. Bertoni*¹, *M.J. Macera*^{2,3}, *V. Mizhiritskaya*^{2,3}, *W. Thelmo*¹, *P. Chandra*³, *A. Babu*^{2,3}. 1) Dept of Pathology; 2) Div of Molecular Medicine & Genetics; 3) Dept of Medicine, Wyckoff Heights Medical Ctr, Brooklyn, NY.

A 55 year old white male with a ten year history of sciatic pain first noticed a bump in his right flank in November 2003. He lost 40 lbs within three months, and the very rapidly growing mass caused increasingly severe pain that eventually incapacitated him. His past medical history was unremarkable. A CAT scan and MRI detected a large mass posterior from heart level to sacrum showing evidence of vertebral involvement with bone destruction. The mass could not be completely removed due to extensive involvement of the fascia. The histological diagnosis of the 21 cm x 14 cm x 6 cm mass was a liposarcoma, myxoid and spindle cell type. Clinically, however, the mass behaved very aggressively. Cytogenetic analysis showed a hyper variable chromosome complement ranging from 64 to 83 chromosomes. Most cells consistently showed several clonal abnormalities consisting of five large marker chromosomes and two ring chromosomes. CGH analysis identified high amplification of chromosome segments 12q14-q21.3(2.5 s.d.) and 5p14-pter (2.0 s.d.), and loss of segments 13q13.2-q22.2 and q31.2-qter. Metaphase FISH using whole chromosome paints of 5 and 12 (wcp5 and wcp12) showed positive labeling for both 5 and 12 paints adjacent to each other on several markers. These findings on FISH signal patterns in view of segmental amplification seen by CGH indicate that the regions 5p14-pter and 12q14-q21.3 were not only present in multiple copies but were co-amplified. One of the oncogenes, MDM2 located at 12q14-15, encodes a protein that inhibits p53 by binding and is implicated in soft tissue sarcomas. Thus the amplification of the chromosome segment 12q14-q21.3 is likely to contribute to increased MDM2 protein that overwhelms p53 regulation. The significance of co-amplification seen between 5 and 12 remains to be seen, whether it is a contributing factor or a coincident. Further molecular studies to investigate the role of chromosome 5p14-ter in this disease are under way. These results emphasize the importance of molecular cytogenetic analysis for all liposarcomas.

Multicolor karyotyping in 50 patients with acute myeloid leukemia. *G. Calabrese^{1,2}, D. Fantasia^{1,2}, P. Guanciali Franchi^{1,2}, M. De Cinque², R. Di Gianfilippo², E. Morizio², G. Perla³, G. Gentile⁴, A. Zatterale⁴, M. Marino¹, A. Spadano⁵, L. Stuppia¹, G. Palka^{1,2}, B. Dallapiccola⁶.* 1) Scienze Biomed/Genetica Medica, Univ G D'Annunzio, Chieti, Italy; 2) Genetica Umana, Ospedale Civile, Pescara, Italy; 3) Ematologia, CSS S. Giovanni Rotondo, Italy; 4) Genetica Medica, ASL 01, Naples, Italy; 5) Dip. Ematologia, Ospedale Civile, Pescara, Italy; 6) CSS-Ist.Mendel, Rome, Italy.

In acute myeloid leukaemia (AML) conventional cytogenetics is unable to establish a karyotype in 20-40% of patients. We analysed by spectral karyotyping (SKY) 40 patients suffering with AML who had a highly rearranged karyotype, i.e. over two breakpoints or at least three rearranged chromosomes, as by conventional cytogenetics. Ten more AML patients with normal karyotype by G-banding were also included in this study. In patients with highly rearranged karyotypes SKY analysis allowed to classify all undefined, or partially defined abnormal chromosomes. Using SKY approach karyotype was refined in 92,5% of patients (37/40). SKY analysis confirmed normal karyotype in the ten patients without apparent abnormalities at G-banding. We classified 41 marker chromosomes (mar), 33 partially identified chromosomes (add), four dicentric chromosomes, and eight cryptic translocations. Chromosomes 5, 7, and 17 were the most frequently rearranged being involved in 70% of cases. We redefined 83 non-specific translocations. Among these a t(5;17)(p10;p10) was observed in two patients. Moreover multicolour chromosome banding (MCB) analysis for chromosomes 5 and 11 disclosed very complex intrachromosomal rearrangements supporting known variability of chromosome-specific breakpoint location and contributing to hunt genes involved in those rearrangements. FISH analysis carried out to confirm SKY/MCB results disclosed involvement of different genes such as AML1 (11 cases), TEL (six cases), MLL (six cases), and deletion of TP53 (13 cases). In conclusion SKY and MCB analyses in AML patients provided a relevant contribute for characterisation of highly rearranged karyotypes unravelling cryptic specific translocations and biological mechanisms of disease.

Breakpoint localization for der(16)t(1;16) in pediatric Wilms tumor using chromosomal and array CGH analyses. *S. Chan, C. Harvard, C. Fawcett, G.P. Jevon, E. Rajcan-Separovic.* Department of Pathology and Laboratory Medicine, Children's and Women's Health Centre of British Columbia, University of British Columbia, Vancouver, Canada.

Background: Wilms tumor is the most common pediatric renal neoplasm and displays various cytogenetic abnormalities. Der(16)t(1;16) is a recurrent rearrangement in Wilms tumor, the breakpoints of which have not been well-defined. We examined the breakpoint (bp) regions of chr 1 and 16 in der(16) using chromosomal and array CGH and correlated the results with the original cytogenetic findings.

Materials and Methods: Three cases of Wilms tumor with der(16) were studied. DNA was extracted from frozen tissue and chromosomal and array CGH were performed. The chromosomal and array CGH analyses were undertaken independently and without the knowledge of the previous cytogenetic findings other than the presence of der(16). The results were then compared and correlated with one another.

Results: Comparison of the conventional cytogenetic findings with the chromosomal and array CGH results showed good correlation of gains and losses in all cases. The results suggested that the chr 1 bp was either in the 1q heterochromatic region (HR) or within the proximal 3.8 Mb of 1q21.1 in all 3 cases. For chr 16, the bp was either in the HR of 16q or within the proximal 0.3 Mb of 16q12.1 in 2 cases. For the remaining case, the bp appeared to be within a 0.6 Mb segment in 16q12.1, located 2.1 Mb from the heterochromatic/euchromatic boundary.

Conclusions: The bp's of chr 1 and 16 in der(16) appear to be within the HR or in the 1st euchromatic band of the q arm a short distance from the heterochromatic/euchromatic boundary. Although the extent of heterochromatic involvement could not be determined in most cases due to the lack of clones in those regions on the array, we demonstrated the extent of euchromatic involvement by using array CGH, and in 1 case, the chr 16 bp was localized to a 0.6 Mb segment in a euchromatic band. Array CGH allows better definition of the bp's and the chromosomal regions involved, facilitating future studies for identifying potential genes involved in Wilms tumor development.

Cytogenetic changes in a L3 lymphoblastic leukemia/Burkitts lymphoma associated with HIV infection. *L. Chen¹, L. Cao¹, Q. Tao¹, F. Haque¹, M. Zhuang¹, HO. Shah^{1,2}, JH. Lin^{1,2}.* 1) Department of Pathology, Nassau University Medical Cent, East Meadow, NY; 2) SUNY Stony Brook, NY.

A 44-year-old man with a history of HIV infection was admitted to hospital with complaints of gum bleeding and generalized weakness. Physical examination revealed petechiae all over skin and splenomegaly without lymphadenopathy. The positive HIV infection was diagnosed six months ago. CD4 count was 772/mm³. The blood count showed RBC of 2.15 x 10⁶/mm³, Hb 6.6 g/dl, Hct 19.5%, platelets of less than 10,000/mm³ and WBC of 23.2 x 10³/mm³ with 28% lymphoblasts. The lymphoblasts exhibited scanty to moderate basophilic cytoplasm containing multiple vacuoles, and nuclei with fine chromatin and conspicuous nucleoli. Cytogenetic study of peripheral blood revealed 46,XY [13]/45,Y,der(X)t(X;18)(p22.1;q12),add(6)(q23),t(8;14)(q24.1;q32),der(13)t(1;13)(q25;q34),add(16)(p11.2),add(17)(q23),18,del(20)(p11.2)[13]. Burkitts lymphoma is most common lymphoma in the patients with HIV infection and often occurs as the initial manifestation of the acquired immunodeficiency syndrome (AIDS). In contrast to other lymphomas associated with HIV infection, Burkitt lymphoma is usually identified in patients commonly having a higher CD4+ T-cell counts (more than 200/mm³), with a short interval between the diagnosis of positive HIV infection and occurrence of lymphoma. Except classic t(8;14)(q24.1;q32), which results in over-expressing MYC gene by juxtaposing the MYC gene adjacent to the promoter of the Immunoglobulin (Ig) heavy chain gene, the possible role of tumorigenesis of other translocations identified in this case, such as t(X;18)(p22.1;q12) and t(1;13)(q25;q34), is not known. Interestingly, the regions of 6q23 and 16p11.2 are the loci of Myb and TLS genes, respectively. Both Myb and TLS have been found to enhance hematopoietic/lymphoid cell proliferation. Additional 6q23 and 16p11.2 in this case likely indicate a positive effect of tumor cell proliferation due to increased gene dosage, which may contribute to the development and progression of Burkitts lymphoma in this AIDS patient.

Oncogene identification in the 1q31 minimal region of genomic gain in retinoblastoma. *T.W. Corson*^{1,2}, *B.L. Gallie*^{1,2,3,4}. 1) Cancer Informatics, Ontario Cancer Institute, Toronto, ON; 2) Molecular & Medical Genetics, University of Toronto, Toronto, ON; 3) Medical Biophysics, University of Toronto, Toronto, ON; 4) Ophthalmology, University of Toronto, Toronto, ON.

The pediatric ocular cancer retinoblastoma is initiated by loss of function of both alleles (M1 & M2) of the prototypic tumor suppressor gene, *RBI*. However, additional genetic alterations (M3 to Mn) are necessary for malignancy. Using comparative genomic hybridization, we and others have previously shown common chromosomal aberrations in retinoblastoma tumors, which may reflect M3 to Mn events. Prominent among these is a region of gain spanning chromosome 1q31, seen in 50% of tumors and likely to harbor oncogene(s) important in retinoblastoma development. To define the minimal common region of gain at 1q31 in retinoblastoma, we used quantitative multiplex (QM)-PCR to screen tumor DNA for gain of 14 sequence tagged sites (STSs) spanning 1q25.2 to 1q32.3. STS marker SHGC-154194 at 1q31.3 showed the most frequent gain in retinoblastoma tumor samples (70%; 39/55). We have thus narrowed the region of 1q31 gain from initially more than 27 Mb, to only 1.2 Mb between markers flanking SHGC-154194. This STS lies within predicted gene *LOC400800*, the mRNA of which is highly expressed in retinoblastoma compared to healthy adult retina by RT-PCR. A slightly expanded 3 Mb region flanking SHGC-154194 contains 16 other known and predicted genes. Of these, only two are highly expressed at the mRNA level in retinoblastoma tumors: *ZNF281* (ZBP99), a zinc-finger transcription factor; and *KIF14*, a kinesin. Moreover, ZBP99 protein is also highly expressed in retinoblastoma. One or more of these highly expressed genes in the minimal region of gain at 1q31.3 may be important in the pathogenesis of retinoblastoma, and may thus be a potential target for novel therapeutics.

Early secondary cytogenetic changes in t(14;18) positive follicular lymphoma. *B.J. Dave, W.C. Chan, D.D. Weisenburger, M.M. Hess, W.G. Sanger.* University of Nebraska Medical Center, Omaha, NE.

The t(14;18)(q32;q21) is the most common recurrent cytogenetic abnormality observed in non Hodgkin lymphomas and is observed in 80-90% of follicular lymphomas (FL). Although the t(14;18) is considered to be the primary abnormality, it is speculated by some that additional genetic alterations are essential to generate a fully malignant phenotype of FL. Complex karyotypes with numerous cytogenetically indefinable changes at diagnosis hampers the delineation of the sequence of alterations which lead to progressive FL. In an attempt to define some of the initial and early secondary changes, we examined the karyotypes of 275 consecutive, histologically confirmed FL containing the t(14;18). Sixty-two cases (23%; 62/275) contained karyotypes that revealed either a t(14;18) alone or had only one other structural or numeric abnormality in the stemline clone. Of these, 16 cases (26%; 16/62) contained the t(14;18) alone and 36 (58%; 36/62) revealed karyotypes with one additional abnormality in a single abnormal clone. Ten cases (16%; 10/62) contained two related abnormal clones where the stemline clone contained only one additional abnormality. Among the 46 cases with one additional abnormality, nine (20%; 9/46) involved chromosome 18, either as a +der(18)t(14;18) or as a +18. Eight cases (17%; 8/46) involved the structural rearrangements of chromosome 1 with 1p36 and 1q44 as recurrent breakpoints. Eight cases (17%; 8/46) contained numeric (+7) or structural changes of chromosome 7, with 7q36 as a recurrent breakpoint; and four cases (9%; 4/46) presented with a structural alteration of chromosome 3 with 3p25 and 3q29 as recurrent breakpoints. Thus, the earliest secondary alterations, as detected by clonal evolution of the karyotypic changes, involved the chromosomes 18, 1, 7, and 3 in descending order, with the recurrent breakpoints being 18q21, 1p36, 1q44, 7q36, 3p25, and 3q29. DNA microarray investigations to determine the possible presence of subtle genomic alterations in the cases with t(14;18) as a sole abnormality, and to delineate the genetic alterations in the recurrent breakpoint regions, will better elucidate the pathogenesis and progression of FL.

Structural alterations of c-MYC, Her-2/neu and CCND1 oncogenes in Langerhans cell histiocytosis. *J. Gogusev*¹, *L. Telvi*², *I. Murakami*¹, *A. Stojkoski*¹, *C. Glorion*³, *F. Jaubert*⁴. 1) INSERM U507, Hosp Necker,; 2) Cytogenetics Laboratory, Hosp St Vincent de Paul,; 3) Orthopedic Surgery Department, Hosp Necker; 4) Pathology Department, Hosp Necker, Paris.

Langerhans cell histiocytosis (LCH) is characterized by the presence of destructive granulomas containing CD1a + Langerhans-like cells, lymphocytes and eosinophils. LOH at several loci as well as DNA copy number changes on chromosomes 1, 5, 7, 9, 13, 16 and 18 were described in LCH lesions of bone. To identify additional changes of pathogenetic importance, we determined the copy number in situ and possible rearrangement of the c-myc, CCND1(cyclin D1), Her-2/neu and p53 proto-oncogenes in LCH of bone. LCH cell suspension was obtained after collagenase treatment of the granulomas (n=7) and smears on slides were prepared. LCH derived stromal cell lines, DOR-1 and Prud-1 were used for comparison. Peripheral blood lymphocytes from a healthy donor were used as an internal control. FISH analysis was performed with centromeric probes for chromosomes, 1, 5, 7, 8, 11, 17 and 22 and locus identifying probes for c-myc, CCND1, Her-2/neu and p53 proto-oncogenes. Expression of mRNAs and proteins was studied by immunocytochemistry and Northern blot. By FISH analysis, amplification of c-myc and Her-2/neu (between 5 and 14 copies per nucleus) was found in 3/7 cases, corrected for aneusomies 8 and 17. Low level of amplification of Her-2/neu was revealed in 2 samples. The fraction of nuclei with c-myc and Her-2/neu amplification ranged between 16 and 23 % and was associated with large bone lytic lesions. In two samples, an increased number of CCND1 copies was revealed in more than 28 % of nuclei. None of the lesions exhibited amplifications of p53 gene. The LCH derived cell lines DOR-1 and Prud-1 showed strong amplification of c-myc (7-23 copies) in 21% of the nuclei, Her-2/neu (9-15 copies) and CCND1(4 to 9 copies) in 21% and 12% of the nuclei respectively. The presented results demonstrate an alteration of c-myc, Her-2/ neu and CCND1 genes copy number in LCH lesions of bone. Structural changes of cell cycle related protooncogenes support the neoplastic origin of a distinct subtypes of histiocytic lesions of bone.

Characterizing complexity: CGH, SKY, and targeted FISH analysis of 15 pancreatic cancer cell lines. A.

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Karyotype analysis can provide clues to significant genes involved in the genesis and growth of pancreas cancer. The genome of pancreas cancer is complex, and G-band analysis cannot resolve many of the karyotypic abnormalities seen. We studied the karyotypes of 15 recently established cell lines using molecular cytogenetic tools. G-banded karyotypes of 10 of these cell lines were previously reported (*Cancer J Sci Am* 4:194). Comparative genomic hybridization (CGH) analysis of all 15 lines identified genomic gains of 3q, 8q, 11q, 17q, and chromosome 20 in 9 or more cell lines. CGH confirmed frequent loss of chromosome 18, 17p, 6q, and 8p. 14/15 cell lines demonstrated loss of chromosome 18q, either by loss of a copy of chromosome 18 (n=5), all of 18q (n=7) or portions of 18q (n=2). Multicolor FISH (Spectral Karyotyping, or SKY) of 11 lines identified many complex structural chromosomal aberrations. 93 structurally abnormal chromosomes were evaluated, to which SKY added new information to 67. Several apparently site-specific recurrent rearrangements were observed. Chromosome region 18q11.2 was recurrently involved in 9 cell lines, including formation of derivative chromosomes 18 from a t(18;22) translocation (3 cell lines), t(17;18) (2 cell lines), and t(12;18), t(15;18), t(18;20), and ins(6;18) (1 cell line each). To further define the breakpoints involved on chromosome 18, YACS from the 18q11.2 region, spanning approximately 8 MB, were used to perform targeted FISH analyses of these lines. We found significant heterogeneity in the breakpoints despite their G-band similarity, including multiple independent regions of loss in a region far proximal to the already identified loss of DPC4 at 18q21. (supported in part by NCI P50CA62924).

Clonal evolution mechanism of Ph negative acute lymphoblastic leukemia involving placement of BCR-ABL fusion gene on other sites than 22q11. *S. Hazourli¹, R. Fetni^{2, 3}, L. Busque², J. Hébert^{1, 2}.* 1) Leukemia Cell Bank of Quebec, Guy Bernier Research center, Maisonneuve-Rosemont Hospital; 2) Department of Hematology, Maisonneuve-Rosemont Hospital; 3) Department of Pathology, MontrealChildren's Hospital.

Amplification of BCR-ABL gene is suggested as one of the mechanism of progression and drug resistance to Imatinib Mesylate in chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL). We have investigated a patient with Philadelphia chromosome (Ph) negative but BCR-ABL fusion gene positive ALL by molecular cytogenetics and Quantitative reverse transcriptase polymerase chain reaction. Fluorescence in situ hybridization (FISH) analysis demonstrated the presence of multiple subclones with one to five copies of BCR-ABL fusion genes located on chromosome 9 and on one to four marker chromosomes. Spectral karyotyping (SKY) identified the marker chromosomes as originating from chromosome 8. The amplification of BCR-ABL fusion was confirmed at the mRNA level by Quantitative RT-PCR. We simultaneously, used centromeric probe for chromosome 8 and the BCR-ABL extra signal dual color probe to detect the intermediate clones of the complex karyotype. This allowed us to identify the mechanism of clonal evolution by successive non-disjunction of the derivative chromosome harbouring the fusion gene. This is the first illustration, to our knowledge, of intermediate clones involving placement of BCR-ABL fusion gene on other sites than 22q11 and successive non-disjunction, leading to amplification of BCR-ABL fusion gene.

CGH-array analysis of primary and metastatic ovarian tumors. *O. Israeli*^{1,2}, *A. Aviram-Goldring*², *E. Friedman*², *J. Korach*³, *G. Ben-Baruch*³, *B. Goldman*¹, *S. Rienstein*¹. 1) Danek Gertner Institute of Human Genetics ; 2) The Susanne Levy Gertner Oncogenetics Unit; 3) Gynecology-Oncology Department, Chaim Sheba Medical Center, Tel Hashomer, Ramat-Gan, Israel.

Invasive epithelial ovarian cancer is the most lethal gynecologic malignancy in the western world. In the majority of patients (~75%) peritoneal metastases are found at the time of primary surgery. Understanding the molecular mechanisms involved in tumor initiation and progression offers hope for developing early detection tools and novel therapeutic approaches. By comparing the genetic alterations in primary ovarian tumors with those in their respective metastases, using Comparative genomic hybridization (CGH) and FISH, we previously showed that genomic alterations in the primary tumors are mimicked in the metastases. In the present study somatic genetic alterations were sought in the same set of tumor-metastases pairs (n=3 primary and 3 metastases) at the gene level by applying the array-CGH method. The results show that most (69%-82%) of the primary tumor alterations can also be detected in the respective metastases, yet the metastases demonstrate additional alterations. One cluster of alterations that was exclusively altered in the metastases, but not in the primary tumors is clustered to 17p12-p11.12. This genomic region was described as an area with a high LOH rate and contains the tumor suppressor gene *LLGL1* and the anti-proliferative gene *PMT1*, which might have a role in ovarian cancer progression. We conclude that there are additional genes that are associated with progression of ovarian cancer that may be detected using the array-CGH technique.

Variant APL t(15;17) originating from two subsequent balanced translocations involving the same chromosomes 15 and 17 and preserving the PML/RARA fusion. *J.A. Jahn¹, J. Scheerle¹, R.J. Meister², R.J. Christie², C.D. Croft¹, S. Wallingford¹, D.W. Heritage¹, P.N. Mowrey¹, A. Meloni-Ehrig¹.* 1) Cytogenetics, Quest Diagnostics Nichols Inst, Chantilly, VA; 2) Arlington-Fairfax Hematology-Oncology, P.C., Arlington, VA.

Acute promyelocytic leukemia (APL) is a myeloid disorder characterized by the t(15;17)(q22;q21). Variants of this translocation have been described. We report a 24-year-old male with APL, who presented with pancytopenia and severe headaches. A fluorescence in situ hybridization (FISH) analysis on bone marrow revealed a variant fusion pattern. The typical t(15;17) FISH pattern for the Vysis dual-color, dual-fusion probe is 1 PML signal, 1 RARA signal, and 2 fusion signals. However, the FISH in this patient showed 2 PML signals, 2 RARA signals, and 1 PML/RARA fusion signal. In order to better understand the origin of this variant pattern, a G-banded chromosome study was performed. The karyotype was: 46,XY,del(15)(q15q22),der(17)t(15;17)(q22;q21)t(15;17)(q15;q21). The combined FISH and chromosome analyses suggested that 2 subsequent translocations had occurred involving the same chromosomes 15 and 17. The first gave origin to the typical t(15;17)(q22;q21) and the second caused the separation of the RARA/PML fusion, which typically occurs on the derivative chromosome 17, while preserving the PML/RARA fusion on the derivative chromosome 15. Although by conventional cytogenetics, we did not see any cells with the typical t(15;17), FISH detected a typical fusion pattern in 5% of nuclei, supporting the occurrence of 2 subsequent events. According to previous reports, the prognostic course of complex and variant t(15;17), in which the PML/RARA fusion on the derivative chromosome 15 is intact, such as in the present case, does not differ from the one in the typical t(15;17). In fact, our patient is presently in remission and doing well after chemotherapy with daunorubicin and treatment with all trans-retinoic acid (ATRA). As the prognostic outcome in APL is associated with the presence of a PML/RARA fusion, it is useful and important to perform both cytogenetic and FISH to better characterize a variant and complex t(15;17).

FISH analysis in a woman without Turner stigmata who has unilateral gonadoblastoma and a 45,X/46,X, idic(Y)(q11.2) karyotype. *S. Kaneko*¹, *S. Tsukishiro*¹, *H. Nishikawa*¹, *A. Arakawa*¹, *K. Suzumori*¹, *S. Young*². 1) OB/GYN, Nagoya City Univ, Nagoya, Aichi, Nagoya, Japan; 2) Cancer and Research Genetics, Univ. of South Carolina School of Medicine.

Gonadoblastoma is a rare ovarian lesion composed of germ cells resembling those of dysgerminoma and gonadal stroma cells resembling those of a granulosa or Sertoli tumor. The presence of Y chromosome material in phenotypic females is associated with a risk of up to 30% of developing a gonadoblastoma in the dysgenetic gonads. We report on a 20-year-old woman with no Turner stigmata, completely female external genitalia with no virilization, who had a gonadoblastoma. At the age of 18 years, she was referred for amenorrhea. Chromosome analysis showed mos 45, X[15]46, X, idic(Y)(q11.2). ish idic(Y)(q11.2)(DYZ3++, SRY++) [15] in lymphocytes. After chromosome analysis and laboratory investigations, she started taking hormone therapy and had menstrual cycles. The follow-up MRI revealed that she had developed a 5cm tumor on her right gonad. Subsequently, r-gonadectomy and a biopsy of l- streak ovary were carried out. Histological investigation revealed a gonadoblastoma in the right gonad and only fibrous tissue in the left gonad. Molecular study by Fluorescence in situ hybridization (FISH) showed that all the tumor cells contained Y chromosome material. Most of the tumor cells had two copies of centromere probes of X and Y chromosomes (DXZ1 and DYZ3) and two copies of SRY. Since gonadoblastoma develops only in patients who have Y-chromosome material and dysgenic gonads, it has been hypothesized and indicated that there is a GBY gene (gonadoblastoma locus on Y chromosome) near the centromere of the Y chromosome. In this case, our finding that all the tumor cells had Y chromosome material also support the possible existence of a GBY.

Genomic imbalances in Korean Brain Tumors By Comparative Genomic Hybridization. *H. Kim¹, Y. Suh², H. Kim¹, Y. Chun¹, S. Park¹.* 1) Institute of Human Genetics, Department of Anatomy, Brain Korea 21 Biomedical Sciences, Korea University College of Medicine, Seoul, Korea; 2) Department of Pathology, Samsung Medical Center, Sungkyunwan University School of Medicine.

Twenty-eight of brain tumors were investigated for chromosomal aberrations by comparative genomic hybridization (CGH). The most frequently detected chromosomal gains involved chromosome 9p21-pter (36%), 7p15-pter (32%), 13q21-q23 (29%), 1q42-qter (25%), 4p15-p15.3 (25%), 4q33-qter (25%), 5p13-pter (25%), 8p21-pter (25%), Yq11.2-12 (25%), Xq25-qter (25%), 4q33-qter (21%), 7q21-q31 (21%), and 18q21-q22 (21%). The frequent sites of copy number decreases were detected at 1p36-pter (18%) and 19q (11%). The recurrent gain and loss of chromosomal regions identified in this study provide candidate regions that may contain oncogenes or tumor suppressor genes respectively involved in the tumorigenesis of brain tumor.

Trisomy 22 as a sole primary abnormality in a patient with unusual acute myeloid leukemia. *M.M. Li^{1,2}, K.C. Kim¹, J. Krause³, M. Rozans^{1,2}*. 1) Hayward Genetics Ctr;; 2) Dept of Pediatrics;; 3) Dept of Pathology Tulane Univ School Med, New Orleans, LA.

It has long been debated whether trisomy 22 is invariably secondary to inversion 16 in AML, or whether it could be a primary aberration in AML. We report trisomy 22 as the sole primary chromosomal abnormality in a patient with an unusual presentation of AML. The patient, a 13-year-old boy, initially presented with fever, weight loss, hepatosplenomegaly, adenopathy, and pancytopenia; his bone marrow was hypercellular with trilineage maturation and mild increase in histiocytes. Lymph node biopsy demonstrated effacement of the normal architecture with T-cells. Cytogenetic studies revealed trisomy 22 as the only abnormality in both the bone marrow and lymph node. FISH study with the CBFβ/MYH11 probe was negative. Skin biopsy showed a normal male karyotype. A maternally inherited, previously undescribed perforin gene mutation was also detected. The tentative diagnoses were LPD, HLH, or lymphoma. Although the patient responded to chemotherapies based on above diagnoses, he continued to show hepatosplenomegaly, pancytopenia, and trisomy 22 in his bone marrow. After 1.5 months treatment, the patients bone marrow showed a striking change: it was now extensively infiltrated by immature myeloid cells, indicating AML. AML-specific chemotherapy was administered and a complete remission was achieved. The trisomy 22 disappeared at remission, but reappeared at AML relapse a few months later with an additional der(17)t(11;17)(q13;p11.2). FISH study with the CBFβ/MYH11 probe at relapse was normal. Trisomy 22 as a sole chromosomal abnormality in leukemia is rare; and, if present, is often associated with AML. AML-M4, in which cryptic inv(16) may be present, accounts for nearly 45% of all trisomy 22-associated AML. However, our case as well as others demonstrate that trisomy 22 can be a sole primary abnormality in AML without a cryptic inv(16). The unusual presentation in said patient could be the result of a possible underlying infection that initially mimicked a lymphoproliferative disorder; or may be associated with the mutated perforin, prompting an initial lymphohistiocytic response.

Molecular Detection of 11p Involvement in 5q- Leukemia Cases. *L. Li¹, J. O'Malley¹, B. Wang¹, L. Guo¹, J. Defeo¹, R. Felgar¹, G. Segel², N. Wang¹.* 1) Pathology, University of Rochester, Rochester, NY; 2) Pediatrics, University of Rochester, Rochester, NY.

The 5q- is a non-random chromosomal aberration associated with MDS and AML. The regions most consistently deleted is 5q31.1. To rule out the involvement of other chromosome(s) in the 5q- formation, chromosome microdissection was applied to the 5q- in a case of myeloproliferative disease. To identify the component chromosomes involved in the 5q- and to confirm the specificity of the probe generated to the 5q-, 10-15 copies of the 5q- were dissected and collected, the DNA was isolated, amplified by DOP-PCR, fluorescent labeled and reversely hybridized to normal metaphase spreads as well as the spreads containing the 5q-. When the 5q- probe was hybridized to normal metaphase spreads, it painted the chromosome segments 5pter->5q13 and 11pter->p13 which indicates that the 5q- involves a translocated segment of chromosome 11pter->11p13. FISH with WCP5 and WCP11 confirmed the reverse FISH results. In a second case of leukemia with an apparently 5q-, FISH with the EGR1 probe(5q31) demonstrated the insertion of EGR1 from chromosome 5q31 to 11p15 instead of a deletion.. The insertion of EGR1 from 5q31 to 11p15 was further confirmed by FISH with the probes specific for the subtelomeric regions of 5p, 5q, 11p, and 11q. In conclusion, our data suggests that gene(s) on 11p may play an important role in some of the 5q- cases and that molecular analysis is crucial for defining structural aberration within this heterogeneous subgroup.

Complex Chromosomal Abnormalities in an Acute Lymphocytic Leukemia. *JH. Lin^{1,2}, N. Motiwala¹, Y. Li¹, E. Seguer¹, L. Chen¹, HO. Shah^{1,2}.* 1) Department of Pathology, Nassau University Medical Center, East Meadow, NY; 2) State University of New York at Stony Brook, NY.

A 64 year-old man revealed leucocytosis and numerous blasts in the peripheral blood on follow-up visit for the treated rectal adenocarcinoma in remission. Eight months prior, a rectal adenocarcinoma was discovered and was managed with combined radiotherapy and chemotherapy with 5 Fluorouracil for three months, followed immediately by an abdominoperineal resection with no evidence of residual carcinoma or regional lymph node metastasis. Peripheral blood flow-cytometry and marrow study confirmed the diagnosis of acute B precursor lymphocytic leukemia FAB L2 (ALL) with positive markers for CD19, CD20, kappa sIg and HLA-DR and negative markers for tdT, CD10, CD13, CD14, CD33 and other T cell markers. Marrow chromosomal studies showed 47,XY,del(3)(q23q25),t(4;7)(q35;q32),t(6;14)(q27;q22),t(11;12)(p13;q13),del(16)(q22),+18,add(19)(q13.3),add(21)(q21). Majority of ALL is primarily present in children under the age of 6 years. Most of ALL seen in adults are of B precursor ALL. ALL is commonly associated with chromosomal abnormalities. The most frequently seen and recently characterized is that of t(12;21)(p13;q22). In this particular case, the chromosomal changes were multiple and complex. There was no specific chromosomal modification, which had been previously elucidated; yet there were individual chromosomes or its segments known to involve in ALL chromosomal alteration, i.e., chromosomes 4, 11, 12, 19, 21. The findings of add(21)(q21) conceivably influences the transactivation of genes by normal AML1 protein triggering some regulatory function of cellular proliferation. The presence of transforming gene, BCL3 in aberrant add(19)(q13) might have set in motion a deregulated expression. It is plausible structural and functional alteration in AML1 protein and BCL3 gene in an intricate interaction with several other chromosomal transfigurations, might have generated disease process resulted in B-ALL in this case.

High-resolution mapping of genomic imbalances in osteosarcoma DNA by use of CGH analysis of microarrays.

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Conventional cytogenetic studies have shown that osteosarcomas (OS) are characterized by complex structural and numerical chromosomal alterations. The application of comparative genomic hybridization (CGH) has led to the rapid accumulation of cytogenetic information on osteosarcoma (OS), however, the limited resolving power of metaphase CGH does not permit precise mapping of imbalances. Array CGH allows quantitative detection and more precise delineation of copy number aberrations in tumors. Unfortunately the high cost and lower density of BACs on available commercial arrays has limited the ability to comprehensively profile copy number changes in tumors such as OS that are recurrently subject to genomic imbalance. In this study, we used high-resolution CGH to investigate recurrent patterns of genomic imbalance by use of DNA derived from eleven fresh and eight formalin-fixed paraffin embedded tumors hybridized to a 19,200-clone cDNA microarray. Chromosomes 1, 6, 8, 17, and X harbored the most imbalances. Ten OSs showed copy number gain or amplification of 17p between the loci bounded by 17p11.2-17p12, and five of these tumors were accompanied by a distal deletion in the region of the p53 gene. Gain/amplification of the X chromosome was verified using interphase FISH using a subset of OS FFPE sections and an OS tissue array. In summary, microarray CGH analysis of the chromosomal imbalances of OS confirm the overall pattern observed by use of metaphase CGH and provides a more precise refinement of the boundaries of genomic gains and losses that characterize this tumor.

Molecular Cytogenetic Characterization of a New Human Papillomavirus Positive Cell Line 16-MT. *E.M. McGhee, P.D. Cotter, J.W. Weier, J.F. Berline, M. Gormley, J.M. Palefsky.* University of California San Francisco, CA.

Anogenital cancers are closely associated with human papillomavirus (HPV). HPV-infected individuals, particularly those with high-grade dysplasias, are at increased risk for cervical and anal cancers. Although genomic instability has been documented in HPV-infected keratinocytes, the full spectrum of genetic changes in HPV-associated lesions has not been fully defined. Only a few karyotypes based on chromosome banding techniques have been reported. We examined four HPV-positive epithelial cell lines, Caski, SiHa, HeLa, and 16-MT, by G-banding and spectral karyotyping (SKY) which revealed multiple numerical, complex and cryptic chromosome rearrangements. Newly analyzed 16-MT cells revealed a complex hypertriploid karyotype with multiple structural and numerical abnormalities. The 16-MT karyotype was interpreted as 78 - 83,XXY,+add(1)(p36.3), +3,+4,+5,+5,+7,+8,+i(8)(q10)x2,+10,der(12),der(13;14)(q10;q10),+15,+16,add(19)(q13.3), +21,+21,-22[cp20]. Multicolor analysis by SKY confirmed and further characterized the anomalies identified by GTG banding. The add(1) was identified as a der(1)(1qter-1q25::1p36.1-1qter), the add(19) as a dup(19) and the der(12) interpreted as a der(11) involving a duplication of chromosome 11 material and rearrangement with chromosome 19. In addition, previously unidentified der(9)t(9;22), der(3)t(3;19) and der(4)t(4;9) were noted. All four cell lines showed losses and gains of DNA due to unbalanced translocations and complex rearrangements of regions containing known tumor suppressor genes. Chromosomal damage in these regions might explain the increased risk of cancer associated with HPV. Furthermore, these data represent the first full characterization of the HPV-positive cell line 16-MT.

Deletion (14)(q21) in a case of precursor B-cell acute lymphoblastic leukemia. *A. Meloni-Ehrig¹, K. Chen¹, J. Jahn¹, S. Suchan¹, J. Scheerle¹, G. Crosby¹, N. Seibel², D. Leitenberg², P. Mowrey¹.* 1) Laboratory of Cytogenetics, Quest Diagnostics Nichols Institute, Chantilly, VA; 2) Department of Hematology and Oncology, Childrens National Medical Center, Washington, DC.

The detection of chromosome abnormalities in precursor B-cell acute lymphoblastic leukemia (pre-B ALL) is a powerful tool for disease management. In fact, some abnormalities are associated with particular prognostic outcomes, such as t(12;21), which correlates with good prognosis, and t(9;22), which is associated with poor prognosis. It is understandable why new abnormalities may be challenging to both cytogenetists and clinicians. We present a case of del(14)(q21) as a sole abnormality in a 5-year-old boy with pre-B ALL. To our knowledge, this has not been reported in pre-B ALL. The G-banded karyotype showed a del(14)(q21) as the only abnormality. FISH using the probes TEL/AML1 and immunoglobulin heavy chain (IGH) showed no rearrangement of TEL/AML1 and a single IGH signal in 20% of interphase cells. Search of the literature yielded one report with a del(14)(q22q24). Lymphoid disorders with del(14q) include B-cell non-Hodgkin lymphomas and chronic lymphocytic leukemia, usually with additional abnormalities. Despite the scarcity of cytogenetic reports on del(14q) in pre-B ALL, several molecular/immunology studies have revealed rearrangements and deletions of the IGH locus in approximately 30% of pre-B ALL, leading to oligoclonality. Although pre-B ALL is thought to arise from the monoclonal expansion of committed B-lymphocyte precursors that are arrested at an immature stage of differentiation, the finding of oligoclonal populations suggests that deletions or rearrangements of the IGH locus are an important factor in this transformation. It is surprising that there are so few cytogenetic reports of del(14q) in this disorder. It is safe to assume that the majority of rearrangements and deletions of the IGH locus may not be detected by routine cytogenetics. Reasons may include low level of abnormal cells or deletions may be too small to be detected. Studies similar to this are warranted to understand the relationship between del(14q) and pre-B ALL and to determine its role in the prognostic outcome.

Cytogenetic Follow-Up of Iranian Breast Cancer Patients. *R. Mirfakhraie*¹, *M. Atri*², *P. Mehdipour*³. 1) National Institute of Genetic Engineering & Biotechnology, Tehran, Iran; 2) Department of Surgery, Cancer Institute, Faculty of Medicine, Tehran, Iran; 3) Department of Medical Genetics, Faculty of Medicine, Tehran University of Medical Sciences.

Breast cancer is the most common malignancy among women. Several factors are involved in initiation and development of breast carcinoma, but epidemiologic evidence point to two areas: Environment and Genetics. Cytogenetic studies in breast have revealed chromosomal alterations in tumor cells. Most breast tumor cytogenetics studies thus far reveal complex chromosomal alterations, and hence it has not been possible yet to associate breast cancer with specific chromosome aberrations, although some specific anomalies are beginning to emerge. Blood cultures from breast cancer patients also have revealed chromosomal changes which are similar to those of tumor cells. Such similarities may be very interesting, since it indicates that factors causing alterations in tumor cells may also operate in human lymphocytes. In the present study we have observed such similarities.

Tumor Genome Anatomy Project (TGAP): Gene Discovery in Tumors in the Genome Era. *S.D.P. Moore*^{1,2}, *P. Dal Cin*^{1,2}, *B.J. Quade*^{1,2}, *S. Herrick*¹, *T. Ince*^{1,3}, *M. Parisi*⁴, *M. Mealiffe*⁴, *D.J. Harris*^{2,5}, *H.L. Ferguson*¹, *S. Strehl*⁶, *A.H. Ligon*^{1,2}, *M. Kleinman*¹, *R. Kucherlapati*^{1,2}, *C.C. Morton*^{1,2}. 1) Brigham & Women's Hosp., Boston, MA; 2) Harvard Medical School, Boston, MA; 3) Massachusetts Inst. of Technology, Cambridge, MA; 4) University of Washington, Children's Hosp. and Regional Center, Seattle, WA; 5) Children's Hosp. of Boston, Boston, MA; 6) Children's Cancer Research Institute, Vienna, Austria.

TGAP is a new initiative to identify genes involved in neoplasia in a high throughput manner taking advantage of naturally occurring human genomic and genetic changes resulting from chromosome rearrangements in individuals with a wide spectrum of neoplasms. The completion of the Human Genome Project is redefining the rapidity of genetic discoveries with potential clinical applications. TGAP will exploit these growing biological resources, and will coordinate an integrated group of researchers with expertise in cytogenetics, molecular biology and oncology. This gene discovery initiative is a natural extension in the application and translation of the complete human DNA sequence.

The strategy of TGAP is first to identify a tumor with novel primary or secondary rearrangement(s) primarily from tumor samples submitted for analysis to the Cytogenetics Laboratory at the Brigham & Women's Hospital. Chromosomal breakpoints are mapped by FISH to discover candidate genes or regions of conserved non-genic sequence. Molecular analysis ensues to pinpoint potential disruption or position effects on gene function.

Preliminary work has uncovered new roles for genes in uterine leiomyomata (UL), acute myelocytic leukemia (AML) and lipomas. A UL with a t(10;17) rearrangement disrupted the histone acetyl transferase (HAT) *MORF* on chromo 10, representing the first HAT to be associated with UL. In addition, an AML patient with a t(11;17)(q23;q12-21) involves a *MLL* and *AF17* fusion where the chromo 17 breakpoint was proximal to *RARA*. Another patient with multiple lipomas was found to have a constitutional rearrangement disrupting *HMGA2* representing the first reported constitutional disruption of *HMGA2*.

Modulation and the Fate of the Cytogenetically Abnormal Myelodysplastic Clone by Azacitidine (AZA C). V.

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Myelodysplastic syndrome (MDS) is a clonal hematopoietic stem cell disorder characterized by progressive bone marrow failure leading to death. Transformation to acute leukemia occurs in 50%. Progression of the disease is associated with accumulation of genomic changes with consequential poor outcome. Aza C has anti-tumor activity through modulation of gene expression and changes in DNA methylation. Treatment with Aza C resulted in significant (60%) improvement in bone marrow function and reduction in transformation to AML (Silverman et al JCO 2002). The aim of this study was to determine whether karyotype changes reflect hematological response and if so, what is the fate of the MDS clone. The Aza C modulation of the karyotype was examined in 159 pts with multiple sequential follow up. Three different cytogenetic/FISH patterns emerged. Group I consisted of 98 pts who had no change in karyotype irrespective whether they presented with normal or abnormal karyotype. They were on Ara C for a mean of 17 months (mo) and hematological response was noted in 70%. Group II consisted of 26 pts who had normalization of the karyotype and achieved a full cytogenetic/FISH reversal within a mean of 15 mo. Hematological improvement was seen in 83%. Group III consisted of 35 pts who either progressed from normal to an abnormal karyotype or presented with an abnormal karyotype and had a clonal evolution. Hematological improvement was noted in 33%. These findings suggest that AZA C can modulate the function of MDS clone in one of three ways: full cytogenetic/FISH remission (21%), lack of cytogenetic progression (54%), and persistence of the cytogenetically abnormal clone with improved bone marrow function (16%). Therefore, patients with a poor or intermediate prognostic score may substantially benefit from AZA C treatment. These chromosomal changes suggest that AZA C may function as a biologic response modifier which alters the malignant phenotype in the absence of cytotoxic effect.

Deletion in the derivative chromosome 9 in a variant Philadelphia (Ph¹) translocation, t(3;9;22). *P. Narasimhan*¹, *A. Babu*^{2,3}, *R. Zeng*^{2,3}, *V. Mizhiritskaya*^{2,3}, *P. Chandra*³, *M.J. Macera*^{2,3}. 1) Forest Hills Medical Center, Forest Hills, NY; 2) Div of Molecular Medicine and Genetics; 3) Dept of Medicine, Wyckoff Heights Medical Ctr, Brooklyn, NY.

A 57 year old female was seen for an elevated white cell count, (WBC 78000 mm³, HB 9.3g/dl, hematocrit 29.0, MCV 80, platelets 486,000 mm³). Differential counts showed promyelocytes, myelocytes, blasts 5%;, eosinophils and basophils all suggestive of CML. The spleen was palpable to about 7 cm below the left cortical margin. Cytogenetic analysis with GTG banding on a bone marrow specimen, revealed a variant Ph¹ translocation, 46,XX,t(3;9;22) (q29;q34;q11.2), in all metaphase cells. FISH analysis for BCR/ABL was performed using a dual color probe set with extra signal (ES) (Vysis). The ES probe is approximately 650 kb on 9q that includes an area well centromeric (upstream) to the argininosuccinate synthetase gene (ASS) to an area well telomeric (downstream) beyond the last ABL exon. Hybridization with this probe in typical BCR/ABL rearrangement produces an extra signal on the derivative chromosome 9, der(9), in addition to the BCR/ABL fusion signal. In this patient, the fusion signal of BCR/ABL on der(22) and corresponding signals on both normal chromosomes 9 and 22 were consistently seen in all metaphase cells. However, the extra signal was consistently absent, indicating a deletion 5' of the ABL breakpoint including the ASS gene on the der(9) chromosome. It has recently been shown that 10 to 15 %; of typical t(9;22) Ph¹ positive CMLs have genomic deletions flanking the breakpoints on the der(9) chromosome. It was also shown that deletions are approximately 3 times more frequent in those cases with variant Ph¹ translocations. The survival rate of patients with a deletion is shorter than that of the patients without a deletion. Therefore, deletions in der(9) is considered to be of prognostic value. The patient has responded remarkably well to Gleevec treatment. She had a normalization of cells with no immature cells present and the spleen returned to normal size. Additional studies are in progress to further delineate the extent of the deletion in the der(9).

Analysis of candidate tumor suppressor genes in prostate cancer pathogenesis. *H. Ostrer¹, M. Salman¹, A. Pearlman¹, A. Grossberg¹, A. Goldsweig¹, J. Melamed¹, M. Zeegers²*. 1) New York Univ School of Medicine, New York, NY; 2) University of Birmingham, UK.

Both germline mutations as well as gross somatic genomic alterations, including allelic loss and gene amplification, contribute to the development of prostate cancer. Genes that are identified within the smallest region of deletion overlap may represent tumor suppressors. To evaluate the status of positional cloning studies for candidate tumor suppressor genes, we performed meta analyses on articles for linkage and allelic imbalances that were published from 1992 to January, 2004. For linkage studies, we identified and mapped the critical breakpoints of recombination for HPC1 between markers, D1S215 and D1S158, a region that contained approximately 33 known and 30 assembly genes, including RNASEL. For HPC2, the critical breakpoints of recombination were between markers D17S974 and D17S953, a region that contained approximately 11 known and 19 assembly genes, including ELAC2. For loss of heterozygosity (LOH) studies, we measured the frequency of events and created smoothed density plots, by estimating marker specific summary LOH together with their corresponding 95% confidence intervals for chromosomes 1, 8, and 17. These LOH intervals were concentrated around RNASEL and MSR1 loci, but not around ELAC2. To further examine whether RNASEL and ELAC2 demonstrate LOH, we used Pyrosequencing to genotype and quantify SNPs within the coding regions. When applied to 20 matched tumor and normal samples, we did not detect any LOH events for either gene, suggesting that such events are rare. To examine for loss of expression of RNASEL, MSR1 and ELAC2 at the protein level, we performed immunocytochemical analysis using peptide-specific polyclonal antibodies against fixed sections of normal prostate and prostate cancer. The resulting analyses showed that RNASEL, MSR1 and ELAC2 were expressed at moderate to high levels in prostate epithelium and had no reduction of expression in 75 prostate cancers. Based on these findings, we exclude these tumors as tumor suppressors and propose other candidates within the critical regions on chromosomes 1, 8 and 17.

Formation of der(19)t(1;19)(q23;p13) in acute lymphoblastic leukemia. *K. Paulsson, A. Horvat, T. Fioretos, F. Mitelman, B. Johansson.* Dept. of Clinical Genetics, Lund University Hospital, Lund, Sweden.

The t(1;19)(q23;p13), which results in a fusion of *TCF3* (previously *E2A*) at 19p13 with *PBX1* at 1q23, is one of the most common translocations in acute lymphoblastic leukemia (ALL). It is seen either as a balanced t(1;19) or as an unbalanced der(19)t(1;19); occasional cases with co-existing t(1;19)- and der(19)-positive clones have also been described. Although it has generally been assumed that the unbalanced form arises from the balanced t(1;19) through loss of the derivative chromosome 1 followed by duplication of the normal homologue, this has never been proven. At least two other mechanisms are possible for the formation of the der(19): an initial trisomy 1 followed by translocation and subsequent loss of the der(1), or a rearrangement taking place in the G2 phase of the cell cycle, with the derivative chromosomes 1 and 19 ending up in separate daughter cells. The different alternatives may be distinguished by investigating markers proximal to the breakpoint in 1q23, because they would be expected to lead to different allelic patterns. Thus, loss of heterozygosity due to the presence of uniparental disomy (UPD) - i.e., both copies of a chromosome being derived from only one of the parents - for chromosome 1 would be present in all der(19)-harboring cases arising via the duplication pathway and in one third of the cases arising via the trisomy pathway, but in none of the der(19) formed via the G2 pathway. In this study, we have used quantitative fluorescent PCR with polymorphic microsatellite markers to investigate chromosomes 1 and 19 in two t(1;19)- and four der(19)-positive ALLs. None of the der(19) cases displayed UPD1, excluding that this aberration arises through the duplication pathway. Because previous findings of cases with co-existing t(1;19) and der(19) clones are difficult to explain if the translocation originated in G2, the present results suggest that unbalanced der(19) may arise from an initial trisomy 1 followed by t(1;19) translocation and loss of the derivative chromosome 1.

Breakage at *FRA11F* may be associated with 11q13 gene amplification. *S.C. Reshmi*¹, *D.W. Schoppy*^{2,3,4}, *R.C. Black*², *X. Huang*¹, *W.S. Saunders*^{2,3,4}, *S.M. Gollin*^{2,3,4}. 1) Dept Human Genetics, GSPH, Univ Pittsburgh, Pittsburgh, PA; 2) Dept. of Biological Sciences, University of Pittsburgh, Pittsburgh, PA; 3) The Oral Cancer Center at the University of Pittsburgh, Pittsburgh, PA; 4) University of Pittsburgh Cancer Institute, Pittsburgh, PA.

Inefficient repair of double strand breaks has been observed in the form of stalled replication forks within areas of the genome exhibiting higher strand flexibility, namely, chromosomal fragile sites. Recent studies have demonstrated that gene amplification in the form of a homogeneously staining region (hsr) may result from breakage at a common fragile site. We previously demonstrated that the 11q13 hsr present in 45% of oral squamous cell carcinomas (OSCC) contains an inverted repeat pattern of amplified *CCND1* flanked by *RINI*. Furthermore, we have evidence supporting a breakage-fusion-bridge (BFB) cycle mechanism for 11q13 gene amplification. Thus, we propose that breakage at the distal, *FRA11F* common fragile site may promote gene amplification of 11q13 in OSCC. In order to characterize the physical map of the *FRA11F* region, we hybridized BACs mapping to 11q13 to normal peripheral blood treated to enhance breakage at common fragile sites. Our data suggest that the *FRA11F* region falls within a 7.7 Mb region. Further studies are underway to determine the precise size of *FRA11F*. However, preliminary results from hybridization of BACs spanning the 7.7 Mb *FRA11F* region to OSCC cell lines with 11q13 gene amplification suggest that breakage at *FRA11F* may be an important step for initiating 11q13 gene amplification in OSCC.

Karyotypic complexity and heterogeneity as potential targets for cancer therapy. *A.V. Roschke¹, S. Lababidi², G. Tonon¹, K.S. Gehlhaus¹, J.N. Weinstein², I.R. Kirsch¹.* 1) Genetics Branch, Ctr Cancer Res, National Cancer Inst, Bethesda, MD; 2) Laboratory of Molecular Pharmacology, Ctr Cancer Res, National Cancer Inst, Bethesda, MD.

We have recently completed a detailed analysis of the chromosomal aberrations present in the drug-discovery panel of 60 human cancer cell lines (the NCI-60) used by the Developmental Therapeutic Program of the National Cancer Institute to screen compounds for anticancer activity, and identified a set of general chromosomal states based on the complexity and heterogeneity of their karyotypes. For the first snapshot of relations between these markers of chromosomal state and drug resistance or sensitivity, we used a 1429-drug subset of the more than 100,000 compounds tested against the cell lines. We have identified groups of chemical compounds that are more cytotoxic toward cancer cell lines with more complex and/or unstable karyotypes. This suggests a novel and complementary approach to current anti-cancer drug discovery. It is a plausible hope that the assessment of chromosomal state of a cancer cell population may provide a future guide for the selection of drugs active against aggressive and intractable cancers.

A Case of a del(21)(q22q22), Trisomy 21 and Rearranged AML1 Gene. *P. Scott*^{1,2}, *S. Hazourli*⁴, *J. Hébert*⁴, *A.M.V. Duncan*^{1,2,3}, *R. Fetni*³. 1) Departments of Human Genetics; 2) Pediatrics; 3) Pathology, McGill University and Montreal Childrens Hospital; 4) Centre de recherche Guy-Bernier, Hôpital Maisonneuve-Rosemont, Montréal, Québec.

Trisomy 21 is the second most frequent trisomy associated with malignant hematopathological disease after trisomy 8. Recent studies associate the deletion del(21)(q22q22) and point mutations of the AML1 gene in hematological malignancies with trisomy 21. We present here a patient with trisomy 21 and thrombocytopenia in transformation. Standard bone marrow cytogenetic analysis revealed trisomy 21 in all cells analyzed with a del(21)(q22q22) in two of the three chromosome 21. Interphase in situ hybridization with the probe AML1/ETO, which covers a 1.3MB region of the sub-band 21q22.1, confirmed the interstitial deletion of band 21q22.1 on one of two chromosome 21 in one of two clones, and on two of three chromosome 21 in the other. Further FISH analysis with probe TEL/AML1, which covers the gene AML1 at the sub-band level of 21q22.1, revealed a single copy of the gene AML1. We analyzed the proliferative activity of the different clones using simultaneous detection of in vitro bromodeoxyuridine incorporation and FISH and showed that the trisomic cell line had a growth advantage over the disomic cell line. We will discuss the relationship between del(21)(q22q22), trisomy 21 and rearrangements of the AML1 gene and their implications in leukemic evolution.

Minimal interval defined on chromosome 7q in uterine leiomyoma. *S.M. Sell, C. Tullis, D. Stracner, C. Song, J. Gewin.* Department of Nutrition Sci, 346 WEBB, University of Alabama at Birmingham, Birmingham, AL.

Uterine leiomyoma are benign, smooth muscle tumors of the female reproductive tract. The tumors are very common and affect approximately 10-15 million women in the United States annually. Uterine leiomyomata are often asymptomatic but they may cause symptoms that range in severity from mild abdominal discomfort to uterine prolapse. Several different chromosomal aberrations are found in the tumor tissue. Because of the common occurrence of this tumor and the potential severity of associated sequelae, research delineating the different molecular subtypes is necessary. Deletions on the long arm of chromosome 7 are thought to be the most common genetic anomaly in uterine leiomyoma. The size of the deletion is variable. This makes it difficult to identify the genes that, upon deletion, contribute to tumor growth. The smallest previously defined interval was greater than 12,000 kb. Using a set of intercalating microsatellite markers we have narrowed a minimal region to an interval of less than 500 kb. The single known gene in this interval is *ORC5L*, a member of the human origin recognition complex. Loss of this gene has previously been described for tumor samples involving larger deletions. It has been hypothesized that loss of this gene might prevent progression to malignancy by retarding the cell cycle. This gene has also been shown to play a role in regulating transcription. Carefully controlled microarray analyses may shed light on this latter possibility.

Genomic analysis of bridge-breakage-fusion events and fragile sites associated with gene amplification in the osteosarcoma cell line MG63. *S. Selvarajah*^{1,3}, *G. Lim*³, *B. Beheshti*⁵, *B. Vukovic*^{2,4}, *J. Squire*^{1,2,4}, *M. Zielenska*^{1,3}. 1) Dept of Pediatric Lab Medicine, Hospital For Sick Children, Toronto, ON, Canada; 2) Department of Cellular & Molecular Biology, Ontario Cancer Institute, Toronto, ON, Canada; 3) Hospital for Sick Children, University of Toronto, Toronto, Ontario (Canada); 4) Department of Medical Biophysics, University of Toronto, Toronto, Ontario (Canada); 5) Faculty of Medicine, University of Toronto, Toronto, Ontario (Canada).

The bridge-fusion-breakage (BFB) cycle is a well-established mechanism for the generation of gene amplification in tumors and model systems. Osteosarcoma (OS) is distinguished by chromosomal instability and high copy number gene amplification, thus the BFB cycle is an attractive mechanism to explain the origins of genomic instability and the evolution of complex abnormal chromosomes carrying amplified DNA (complicons). MG63 is a representative human osteosarcoma cell line that was shown to have acquired complicon structures, comprising equally-spaced intrachromosomal amplifications arising from chromosomes 6, 8 and 9. Detailed analysis of this cell line showed that dicentric chromosomes and inverted duplications were present at a high frequency. In addition, FISH analysis using mBAND subregional cytoband paint probes of the complicon, showed a pattern of interspersed amplification of regions 6p21, 8q24 and 9p22. Moreover, inverted duplications were found within amplicon subunits suggesting that BFB was associated with the generation of gene amplification from these three regions in this cell line. Known intermediates of the BFB cycle, such as dicentrics, anaphase bridges and chromatin strings were also quantified and observed to occur at high frequency. The precise regions of co-amplification were then mapped by high-resolution array CGH and FISH. Interestingly, the amplicon boundaries were found to be flanked by regions containing fragile sites, suggesting they may have been involved in generating precursor amplicon structures. In summary, this study provides further support for the role of BFB mechanism and fragile sites in gene amplification and chromosomal instability in OS.

Retinoblastoma in a newborn baby with a heterozygous Rb gene deletion. *HO. Shah^{1,2}, P. Koduru³, A. Karnik¹, B. Miller¹, V. Diaz-Barrios¹, Y. Zhang¹, M. Zhuang¹, J. Sherman^{1,2}, JH. Lin^{1,2}.* 1) Pathology & Lab Cytogenetics, Nassau Univ Medical Ctr, East Meadow, NY; 2) State Univ. of Stony Brook; 3) Cell Genetics, Northshore University Hospital, Manhasset, NY.

A full-term baby boy was born to a gravida 1, para 1, 20-year-old woman. Shortly after pulmonary hypertension was diagnosed and treated accordingly. The baby exhibited facial asymmetry, swollen right sternocleidomastoid muscle and dysmorphic bulging eyes. Funduscopic findings and a well-defined soft mass along the posterior aspect of right globe by CT scan and MRI established the clinical diagnosis of retinoblastoma in the right eye. Peripheral blood chromosomal analysis revealed 46,XY,del(13)(q12.3q21.2). Parental blood samples for chromosomal studies showed a structurally unremarkable normal chromosomal makeup in both parents. Fluorescence in-situ hybridization study with LSI-13 (RB1, Vysis) was performed on metaphase spreads of the child and of both parents. Normal signal pattern was detected in both parents. In the child, the target region (RB1 region) was deleted in the abnormal 13 only. These findings suggest that a de-novo deletion in one 13 retinoblastoma region leading hemizyosity for this region in the child. At the age of one and one half months, the right eye was enucleated which was followed by chemotherapy. Patient is doing well at the age of 5 months. Retinoblastoma (RB) is the most common intraocular tumor of children and usually diagnosed between the ages of 16 months and two years. About 60% of RB is sporadic and the other 40% are inherited. The retinoblastoma gene (Rb) locates in chromosome 13q14. Persons who carry one of mutant alleles in association with predispositional factors have a risk of 80-90% chances to develop RB. The Rb protein produced in this gene is inactivated when both alleles are mutated rendering cellular proliferation and thus formation of neoplasms. Since this case displays de-novo deletion of one single retinoblastoma gene in chromosome 13, this must have been followed by a mutation in the other allele triggering the development of retinoblastoma in the child.

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Inferring the location of tumor suppressor genes and oncogenes by modeling array CGH data. *A. Sterrett, F.A. Wright.* Biostatistics, University of North Carolina, Chapel Hill, NC.

Comparative genomic hybridization (CGH) can detect partial chromosomal deletions and amplifications, which may contain genes associated with the development of tumors. The use of array CGH in the literature has increased dramatically in the past year, yet many of these studies do not use a statistical approach to search for genes associated with tumorigenesis. Statistical modeling of array CGH data can improve genomic searches by accounting for sporadic background loss and correlations between neighboring markers. We use Hidden Markov Models to calculate likelihoods from array CGH data, from which we can infer the location of tumor suppressor genes and oncogenes. Specifically, we define three hidden states (decreased copy number, normal copy number, and increased copy number) underlying the raw intensity ratios or a discretized transformation of the intensity ratios. Our model accommodates unequally spaced markers and missing observations. Additionally, it is possible to allow the model parameters to depend on covariates, such as tumor stage, in order to increase precision. We demonstrate the use of this statistical methodology through a recent array CGH study.

Cell death by chromosome elimination: Characterization of drug induced chromosomal fragmentation. J.B.

*Stevens*¹, *S. Savasan*², *G. Liu*¹, *S.W. Bremer*¹, *M. Atanasovski*¹, *W. Xu*¹, *T. Trivedi*¹, *C. Ye*⁵, *H.H. Heng*^{1, 3, 4}. 1) CMMG, Wayne State University, Detroit, MI; 2) Hematology/Oncology, Childrens Hospital of Michigan, Detroit, MI; 3) Karmanos Cancer Insititute, Detroit, MI; 4) Department of Pathology, Wayne State University, Detroit, MI; 5) SeeDNA Biotech Inc, Windsor, ON.

The subject of cellular death is of great importance in the regulation of development and the process of carcinogenesis. Here we describe a novel form of mitotic cell death in which chromosomes are eliminated through fragmentation into many small pieces. Chromosome fragmentation refers to a phenomenon in which chromosomes are cut into progressively smaller pieces. This can result in mitotic figures where cut and uncut chromosomes coexist within the same figure. Under drug treatment, the degree of chromosomal fragmentation increases until all chromosome morphology is lost and the cell dies. Occasionally, some fragmented chromosomes can form micronuclei if the cell can complete telophase. Even though there are some morphological similarities between chromosomal fragmentation and S-phase PCC (Premature Chromosome Condensation), the key difference is that fragmentation takes place only in mitotic cells. Unlike classical apoptosis, chromosomal fragmentation does not depend on p53 status and does not require caspase activation. Fragmentation is TUNEL negative, however there are DNA strand breaks present as indicated by -H2AX staining. Additionally multiple color SKY has shown that certain chromosomes are more frequently found to be fragmented, indicating that chromosome fragmentation is an orderly event. Treating cells with clinically significant drugs such as doxorubicin and methotrexate can consistently induce chromosome fragmentation. It is therefore possible that the process of chromosomal fragmentation could provide a potentially important biomarker to document levels of cell death in tumors and determine drug toxicity.

Chromosomal aberrations in Korean cervical squamous cell carcinoma cell lines by CGH and array-CGH. K.

Uhm¹, K.Y. Oh², S. Chun³, S.C. Kim³, K. Jeong³, J.I. Kim³, S. Park⁴, S. Park¹. 1) Institute of Human Genetics, Department of Anatomy, Brain Korea 21 Biomedical Sciences, Korea University College of Medicine, Seoul, Korea; 2) Department of Obstetrics and Gynecology, Eulji Medical College, Daejeon, Korea; 3) Department of Obstetrics and Gynecology, College of Medicine, Ewha Womans University, Seoul, Korea; 4) Department of Anatomy, College of Medicine, Ewha Womans University, Seoul, Korea.

The array-comparative genomic hybridization (CGH) provides a method to measure the DNA copy-number changes quantitatively at an extremely high resolution and to map them directly onto the complete linear genome sequences. The non-random chromosomal DNA aberrations were analyzed in cervical squamous cell carcinoma cell lines from Korean women, SNU-17, SNU-682, and SNU-902 using CGH and array-CGH. Gains of 5p, 5q22-q23, 8q11.2-q12, 14q21-qter, and 20q were found in all three cell lines. Gains on chromosome 3q, 7p13-pter, 9p22-pter, 9q21-qter, 15q21-q22, 17q22-qter, 18p11.3-pter, 18q11.2-q21, 19p13.3-pter, and 19q13.2-q13.3, with losses on 4p14-pter, 10p11.2-p13, 10q24, and 21q were observed in two of three cell lines. The results of CGH in cervical squamous cell carcinoma cell lines were similar to array-CGH analysis. Data about chromosomal aberrations of Korean squamous cell carcinoma cell lines in this study could afford very useful basic information for the development of diagnostic and therapeutic modalities targeting the abnormal genes associated with uterine cervical cancer in Korea.

The use of culture mitogens in B-cell disorders. *D. Van Dyke*^{1,2}, *A. Wiktor*^{1,2}. 1) Dept Lab Medicine & Pathology, Mayo Clinic, Rochester, MN; 2) Henry Ford Hospital, Detroit, MI.

A low mitotic rate and a poor response to mitogens have hampered cytogenetic analysis of B-cell disorders. By identifying optimal cell culture conditions, the likelihood of detecting cytogenetic abnormalities may be increased. From a series of 1885 samples with a known or suspected B-cell disorder, we compared the detection rate of abnormalities in unstimulated and mitogen stimulated cultures (B-cell lipopolysaccharide plus pokeweed). We excluded 30/1436 (2%) bone marrow, 25/94 (27%) peripheral blood, and 79/355 (22%) lymph node samples that failed to produce metaphases. We also excluded cases with fewer than analyzable 5 cells from a culture (68 marrow, 9 blood, and 40 lymph node samples). A clonal abnormality was identified in 168/1338 (13%) marrow, 6/60 (10%) blood, and 38/236 (16%) lymph node samples. Analysis of cells from B-cell mitogen cultures increased the yield of abnormalities in marrow aspirate samples by 13%, from 146/1338 (11%) to 168/1338 (13%). For lymph node samples, B-cell mitogenized cell cultures were of no significant added value in detecting chromosome abnormalities. The sample size of abnormal peripheral blood samples was too small to be informative. Of the 213 abnormal cases, at least 24 (11%) were identified solely in B-cell mitogenized culture, and in another 15 cases (7%) the B-cell cultures served to confirm a non-clonal abnormality seen in the unstimulated culture. Of the 24 cases that only had an abnormality in the B-cell cultures, 21 had the abnormal cell population in fewer than 30% of cells; additional studies may help determine whether B-cell mitogens are useful to reveal an emerging population. Of these 24 cases, 6 would have exhibited a normal FISH pattern using our current B-cell FISH probe panels, five had simple +12 or 11q+, and 13 had additional aberrations that would not have been observed by interphase FISH alone. Although interphase FISH is useful to identify common abnormalities associated with B-cell disorders (Wiktor & Van Dyke, 2003 ASHG abstract and in press), a B-cell mitogenized culture does provide a modest improvement in the chromosome aberration detection rate when a B-cell disorder is suspected.

Cytogenetic Analysis and FISH of terminal deletion of the long arm of Chromosome 9 in one patient with Acute Promyelocytic Leukemia. *G. Vasquez-Palacio¹, O. Botero-Galeano¹, A. Posada-Sanchez², T. Teknirat³, M. Sierra-Sanchez², N.E. Durango-Calle¹, C.M. Cristancho-Salgado¹, J.C. Herrera-Patino¹, F. Quintero-Rivera^{1,4}, J.L. Ramirez-Castro¹.* 1) Medical Genetic Unit, School of Medicine, University of Antioquia, Medellin, Colombia; 2) Children's Hospital, Hospital Universitario San Vicente de Paul, Medellin, Colombia; 3) Department of Genetics and Development, Columbia University, New York, NY; 4) Center for Human Genetic Research, Massachusetts General Hospital, Harvard Medical School, Boston, MA.

Deletions of the long arm of 9 del (9q22-ter) are rare aberrations specifically found in acute myeloid leukemia (AML). Yamamoto et al 1999, reported the first case of acute promyelocytic leukemia (APL) with a terminal 9q deletion as sole abnormality. Here we describe the second case with the same aberration. The patient, an eleven-years-old girl with APL. The laboratory studies (Hb) of 88 g/L, 12x10⁹/L, count neutrophils 12%, lymphocytes 15%, blasts 71%. Cell suspension immunophenotypic studies were performed using the following antibodies: CD3, CD 13, CD19, CD33 and myeloperoxidase (MPO) positive. Chromosomal analysis by the Giemsa R banding technique and FISH using LSI BCR/ABL Vysis probe was performed on short-term cell cultures from bone marrow. Average of 50 metaphases per patient. Normal chromosome 9, and del (9)(q22) were detected. Deletions of 9q are described to be about 3 to 4% in AML, especially in Myeloid Leukemia M1 and M2. Sole 9q terminal deletions, are less common than interstitial ones and predominant involve q21~q22 band. The ABL proto-oncogen has been localized in this region on chromosome 9, this gene is important for understanding the genetic mechanisms involved in leukemogenesis of LMC. However, a recent study suggests that patients with APL and del deletion (9)(q22) shows a relatively good prognosis.

Bridge-fusion-breakage (BFB) as a driving force for karyotypic evolution of prostate cancer (CaP). *B. Vukovic*^{1,2}, *B. Beheshti*², *G. Lim*², *J. Bayani*², *S. Watson*³, *W. Lam*³, *J. Squire*^{1,2}. 1) Medical Biophysics, Ontario Cancer Institute, Toronto, Ontario, Canada; 2) University Health Network, Toronto, Ontario, Canada; 3) BC Cancer Research Centre, Vancouver, British Columbia, Canada.

BACKGROUND: Telomere erosion and ploidy changes were found to be early events in prostate cancer progression, but there is no evidence of a BFB mechanism generating the observed genetic changes. An ongoing chromosomal instability (CIN) was observed in DU-145 cell line with generation of novel numerical and structural aberrations upon cell division. To investigate BFB as a mechanism underlying the observed CIN process, molecular cytogenetic methods were used to study the karyotypic evolution in prostate cell lines. **METHODS:** Anaphase bridge frequency was determined using immunocytochemistry (ICC) with CREST antibodies for kinetochore identification. Dicentrics were analyzed using FISH and their functionality evaluated through examination of CENPA/B status. Progression of structural rearrangements concordant with BFB was examined using spectral karyotyping (SKY) and multicolor banding (mBAND) methods. Comparison of mBAND to aCGH profiles allowed more precise mapping of regions highly prone to BFB induced breakage. **RESULTS:** DU145 and PC3 were found to have high frequency of anaphase bridges with involvement of dicentrics seen at much higher frequency in comparison to control lines (PrEC-T). Stabilization of clonal dicentrics was concordant with inactivation of one of the centromeres. Detailed examination of karyotypic rearrangements has shown complex structures and resulting derivatives concordant with repeated fusion and breakage of chromosomes. The breakage was found to be non-random and clustered at specific points along the chromosomes. The breakpoints often mapped to amplicon boundaries and regions of high fragility in the genome. **DISCUSSION:** BFB was found to be one of the mechanisms that contributed to CIN previously documented in prostate cancer cell lines although there were indications of other mechanisms at play. Analysis of telomere length in these lines suggested further telomere dysfunction as a source of observed BFB. Implications of these findings in CaP progression will be discussed.

Trisomy 22 is an important secondary change associated with AML M4 (AML-M4) with inv(16)(p13q22). D. Wei¹, K.H. Ramesh¹, V.R. Pulijaal¹, M. Zohouri¹, D.T. Walsh¹, R.A. Conte¹, A.R. Deshikar¹, T. Zhou¹, H. Ratech², X.Y. Yang², C. Sands², M. Ramirez², L.A. Cannizzaro¹. 1) Department of Pathology, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY.10461; 2) Hematology-Oncology, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY.10461.

A 36-year-old female with anemia and elevated white blood count was diagnosed with AML-M4 in June 2001. Flow cytometry showed CD2+, CD11b+, CD11c+, CD13+, CD14+, CD15+, CD33+, CD34+, CD38+, HLA-DR+. Cytogenetic analysis of bone marrow (BM) at this time showed a 46,XX,inv(16)(p13q22)[20] karyotype. She achieved complete remission in May 2002. In Jan. 2004, BM morphology showed normocellular marrow (40-60%) with relative erythroid hyperplasia and maturation arrest in the myeloid series. Flow cytometry was 14-19% CD13+, CD33+, CD34+, CD38+, CD117+ myeloblasts. Cytogenetic and FISH analyses in Jan 2004 revealed a karyotype of 47,XX, inv(16)(p13q22),+22 [16]/46,XX[4]. ish inv(16)(p13q22)(pcp16q sp)(wcp22+). These results were consistent with AML in relapse. She achieved clinical and cytogenetic remission in Apr. 2004. Inversion 16 (p13q22) has been reported in about 2% of AML and is specifically associated with the M4E0 subtype and a favorable prognosis. In about 40% of AML cases with inv (16), trisomy 22 can be found as an additional abnormality. It has been suggested that trisomy 22 occurs as a secondary change during clonal evolution of AML with inv(16). It has been shown that about one third of the patients with molecular evidence of CBF/MYH11 fusion do not have cytogenetic changes of inv (16). The literature and our observations suggest that trisomy 22 is an important secondary change in AMML or AML with a monocytic component and inv (16). Inv (16) can be difficult to recognize, particularly in poor cytogenetic preparations. Therefore, the presence of trisomy 22 should alert the hematologists, cytogeneticists and molecular biologists to perform appropriate tests such as FISH or other molecular tests to confirm the evidence of inv (16), which has an important prognostic impact in the clinical management of AML.

Concurrent presence of t(10;11)(p12;q23) and t(11;19)(q23;p13.3) in an infantile AML case. *S. Wu^{1,2}, G. Lu¹, J. Dang¹, XR. Chen¹, J. Quinn².* 1) Dept of Pathology and Lab Medicine; 2) Dept of Pediatrics, Children's Hospital Los Angeles, Keck Med School, University Southern California, Los Angeles, CA, USA.

The translocations t(10;11)(p12;q23) and t(11;19)(q23;p13.3) are closely associated with human acute leukemia with the former predominantly presented in de novo AML(m5a) and the latter occurred in both acute myeloid and lymphoid infantile leukemia. To the best of our knowledge, these two translocations have yet to be reported in the same patient. We report here an infantile leukemia case with concurrent presence of two translocations: t(10;11)(p12;q23) and t(11;19)(q23;p13.3). This is a 3.5-month old Latino infant who initially was presented with leukemic skin involvement and hepatosplenomegaly with the bone marrow showing 31% myeloblasts. The bone marrow cytogenetic analysis showed concurrent presence of t(10;11) and t(11;19) which were confirmed by FISH studies. The patient was treated with Idarubicin, Fludarabine, Ara-C, and L-asparaginase and received a short period of remission following a recurrent leukemia cutis and involvement of testis. He was then administered reinduction therapy with mitoxantrone and Ara-C and awaits recovery. The correlation between the unique leukemia cutis and testis and the concurrent presence of t(10;11) and t(11;19) in this patient is worth further investigation.

Direct visualization of hybrid tumor cells in human cancer by FISH: Donor Y chromosome in renal carcinoma cells of a female BMT recipient. *Y. Yilmaz*^{1,4}, *R. Lazova*^{2,4}, *M. Qumsiyeh*^{1,4}, *D. Cooper*^{3,4}, *J. Pawelek*^{2,4}. 1) Dept Genetics, Yale Univ Sch Medicine, New Haven, CT; 2) Dept Dermatology, Yale Univ Sch Medicine, New Haven, CT; 3) Section Medical Oncology, Yale Univ Sch Medicine, New Haven, CT; 4) Yale Cancer Center, Yale Univ Sch Medicine, New Haven, CT.

There are more than 25 reports of spontaneous tumor hybridization in animals, and several link hybridization with metastasis. To investigate this in humans, we probed a primary renal cell carcinoma from a female patient who, 2 years prior to detection of the tumor, received a bone marrow transplant (BMT) from her 15 year old son. Karyotype/G-banding analyses revealed that some of the tumor cells contained a chromosome 17 trisomy (T17). This provided an opportunity to use FISH to visualize cells in fixed tumor sections containing both the T17 tumor marker, and the Y chromosome BMT donor marker. Combining H&E staining with dual-label FISH, these markers were detected together in individual nuclei of carcinoma cells, providing direct genetic and morphological evidence that such cells were BMT-tumor hybrids. In a single tumor section nearly 50 cells contained both the Y chromosome and a T17. Further, when a sequential section was stained for beta 1,6-branched oligosaccharides (a metastasis-associated trait, associated with poor outcome in breast, lung, and colon carcinomas) in combination with FISH, 80% of the carcinoma cells staining for these sugars also contained the Y chromosome, indicating that BMT-tumor hybrids were the predominant source of the sugars in this tumor. To our knowledge, this is the first direct visualization of hybrid tumor cells in human cancer. That these cells produced beta 1,6-branched oligosaccharides indicates they may have had increased metastatic potential. The results suggest that tumor hybridization merits renewed consideration as an underlying factor in human metastasis. Continued analyses of malignancies in BMT recipients should be a fruitful approach to this important question.

Chronic Lymphocytic Leukemia: A Prospective Study Using FISH, CD38, and ZAP-70. *E. Zafer, S. Abbasi, B. Cheson, M. Ozdemirli, D. Hartmann, J. Meck.* Georgetown U Hosp, Washington, DC.

CLL shows a highly heterogeneous clinical course with some patients not requiring therapy for years, while others progress rapidly. Thus, alternate molecular, cytogenetic and clinical factors have been sought as possible prognostic factors. Cytogenetic findings are a strong predictor of outcome. Deletions of ATM, p53, and perhaps trisomy 12, are associated with an unfavorable outcome, whereas deletion 13q14 is associated with a better outcome. ZAP70 and CD38 expression have been shown to predict response to therapy and survival, and correlate with a more aggressive clinical course and shorter median survival. Georgetown U. Hospital is conducting a prospective study of CLL patients in whom chromosomal aberrations by FISH, CD38 and ZAP70 status are being examined. Since Jan 2003, a total of 44 B-CLL patients have been studied. Metaphase chromosome analysis is difficult and unreliable, making FISH an important tool in CLL. Probes to p53, ATM, 13q14, 13q34, and 12 centromere were used. FISH results were abnormal in 27/44 (61%) cases: 17 with del13q14 (63%), and 7 trisomy 12 (26%). Deletions of ATM and/or p53 were infrequent (11%), and never seen as sole events. Our results show a clear correlation between two good prognostic factors: deletion 13q and absence of CD38. Thirty-two cases were CD38- (73%), and 12 were CD38+ (27%). Absence of CD38 was associated with a higher incidence of chromosomal abnormality: 20 (63%) CD38- cases had abnormal and 12 (37%) CD38- cases had normal FISH results. Among the 20 CD38 negative patients with abnormal FISH results, 15 (75%) had loss of 13q, 3 (15%) had +12 and 2 (10%) had multiple abnormalities. ZAP70 expression was evaluated in 26 cases by immunohistochemistry (IHC) and/or flow cytometry: 16 were positive (62%) and 7 (27%) negative, and 3 discrepancies between IHC and flow data. Among the 16 ZAP70+ cases, 7 were normal (43%), 6 were del13q (38%) and 3 were +12 (19%). Among the 16 ZAP70+ cases, 11 of them were CD38- (69%), 5 of them were CD38+ (31%). The prognostic importance of various combinations of FISH, ZAP70 and CD38 will be monitored in larger numbers of patients with respect to patient survival.

Nonrandom duplication of the active and inactive X chromosomes in non-Hodgkin lymphoma (NHL). P.

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The gain of one or more X chromosomes is one of the most common acquired numerical chromosome aberrations in NHL and occurs in both male and female patients. In female patients either the active or inactive X may be gained in a random or nonrandom fashion, whereas the only active homologue must be duplicated in male patients. However, a supernumerary active X could subsequently also become de novo inactivated in both sexes. Previous molecular genetic evidence that derives from the semi-quantitative analysis of differentially methylated polymorphic X chromosome sequences suggested that the acquired X chromosomes are and remain always active in male patients, whereas both the active and inactive X may be randomly duplicated in females (McDonald et al, *Genes, Chromosomes & Cancer* 28:246;2000). However, reliable quantification is difficult, particularly in cases with multiple copies of X chromosomes. In addition, de novo hypermethylation of particular regions that are commonly used for such analyses may also impede the interpretation of the respective results. To overcome these problems, we have developed a dual-color DNA/RNA FISH assay that enables the simultaneous enumeration of active and inactive X chromosomes on a single cell level (see abstract by Haas et al). We used this assay to study 29 methanol/acetic acid-fixed NHL cell samples. They comprised 18 male (9 in the hypo- to hyperdiploid and 9 in the pseudotri- to pseudotetraploid range) and 11 female patients (7 with three X and 4 with four X chromosomes). Except for one case, which most likely was a Klinefelter syndrome with a constitutional XXY, all X chromosomes were active in male patients. This was also the case in all females with three X, whereas those with four X had duplicated both the active and inactive X chromosome. These results concur with those obtained in hyperdiploid childhood ALL and imply that in all instances a single nondiscjunction event leads to the maldistribution of chromosomes irrespective of the ploidy range.

Characterization of breakpoints by fluorescent in-situ hybridization (FISH) analysis in a patient with t(14;18) (q32;q21) and CD- lymphoplasmacytic lymphoma. *M. Zohouri¹, L.A. Cannizzaro¹, V.R. Pulijaal¹, R.A. Conte¹, D.T. Walsh¹, D. Wei¹, A.R. Deshikar¹, T. Zhou¹, T. Hebert¹, X.Y. Yang¹, H. Ratech¹, E.W. Friedman², K.H. Ramesh¹.* 1) Pathology, Montefiore Medical Center, Albert Einstein Col. Med., Bronx, NY; 2) Hematology-Oncology, Montefiore Medical Center, Bronx, NY. 10467.

A 71 year old female with a 10 year history of CLL presented with confusion, headache, and epistaxis. CBC showed Hct of 26%, WBC of 16,200, total protein of 11.8g/dl, IgM of 8160mg/dl, viscosity 5cp, and a thrombin time of 27/2s. CAT scan showed adenopathy but no splenomegaly. Bone marrow (BM) was hypercellular (80%-90%) and was infiltrated in a diffuse and infiltrative pattern by small lymphocytes with plasmacytoid features (70%). Normal trilineage hematopoiesis was reduced. Megakaryocytes were decreased and hypolobated. There were no granulomas. Rare lymphoid cells containing globules that stained focally positive with PAS-D were present. Flow cytometry interpretation was: abnormal cells comprising 23% of the total population, CD5-, CD10-, CD19+, CD20 (moderately bright)+, CD22+, CD23-, FMC7 (partial)+, CD56-, CD138-, monoclonal IgM+, IgD+, KAPPA (bright)+, aberrant mature B-cell phenotype. Based on BM morphology and flow cytometry, a diagnosis of lymphoplasmacytic lymphoma was established. Cytogenetic analysis of BM revealed a 46,XX,t(14;18)(q32;q21) [6]/46,XX [14] karyotype. The t(14;18) is usually seen in follicular lymphoma and diffuse large B-cell lymphoma (DLBCL). FISH analysis with the IGH/BCL2 DNA probe showed an unusual signal pattern; the breakpoint in 18q21 was found to be proximal to the expected BCL2 breakpoint, which resulted in the transpositioning of the entire BCL2 locus to the 14q32 chromosome region. Whereas the breakpoint on IGH occurred at the IGH J segments breaking apart the IGH probe, thereby resulting in partial transpositioning of IGH sequences on to 18q21. The FISH results showed that BCL2 was intact, and does not support follicular or DLBCL in this patient. This case shows the importance of performing FISH analysis where t(14;18) (q32;q21) has been reported by cytogenetics analysis.

Genomic Evolution in Chronic Lymphocytic Leukemia. *L. Zou, L. Li, M. Shaver, J. Lian, J. O'Malley, L. Defeo, N. Wang.* Pathology, University of Rochester, Rochester, NY.

To identify the sequential genetic alterations associated with the onset and progression of CLL, 48 cases were analyzed using both G-banding and Fluorescence In situ Hybridization (FISH) analyses with a panel of probes specific for the regions of 11q22.3(ATM), 12cen, 13q14.3(D13S319), and 17p13.1(p53). Fourteen cases were normal by both G-banding and FISH analyses. A single genomic aberration was identified by FISH in 12 cases: 8 cases (66.7%) with a del(13)(q14.3) and 2 cases (16.7%) with a +12. In contrast, G-banding detected only two cases with an aberration. Twenty cases were detected with more than one genetic alteration by combined G-banding and FISH analyses. Four cases were found to have multiple karyotypic aberrations by G-banding but with no aberrations detected by FISH. Out of 16 cases with aberrations detected by FISH, del(13)(q14.3) was identified in 13 cases, del(11)(q22.3) in 8 cases, +12 in 6 cases, and del(17)(p13.1) in 2 cases. Out of the 13 cases with del(13)(q14.3), 7 cases with an additional aberration of del(11)(q22.3), 3 with an additional aberration of +12, 2 with del(17)(p13.1), and 1 with +11. With the exception of one, all of the 8 cases with del(11)(q22.3) are associated with del(13)(q14.3). Out of the 6 cases with +12, 3 cases are associated with del(13)(q14.3), 2 with del(17)(p13.1), and 1 with a +11. In one case, initially, only del(13)(14.3) was detected, while 6 months later del(11)(q22.3) was detected in 74% of the cell population. In summary our data suggests that del(13)(q14.3) is an initial genetic aberration associated with CLL onset while del(11)(q22.3), +12, and del(17)(p13.1) may take place sequentially afterward during the progression of the disease. Furthermore, FISH analysis with CLL panel probes can highly facilitate the detection of genetic aberrations associated with CLL and increase the sensitivity by extending the analysis to the nondividing cell population. However, G-banding should be applied as well for the detection of other chromosomal aberrations.

Lower Cancer Risks in Amsterdam-I Criteria Families Without Mismatch Repair Deficiency. *K. Rabe¹, G. Petersen¹, R. Haile², G. Casey², M. Aronson³, B. Bapat³, J. Hopper⁴, J. Jass⁴, L. LeMarchand⁵, J. Grove⁵, J. Terdimann⁶, P. Conrad⁶, J. Potter⁷, P. Newcomb⁷, G. Moslein⁸, S. Thibodeau¹, L. Boardman¹, D. Seminara⁹, N. Lindor¹.* 1) Mayo Clinic,Rochester,MN; 2) USC,LA,CA; 3) U of Toronto,Ontario; 4) U of Melbourne,Aust; 5) U of H,Honolulu,HI; 6) UCSF,CA; 7) FHCRC,Seattle,WA; 8) Dusseldorf,Germany; 9) NCI,Bethesda,MD.

Background: The Amsterdam I Criteria (AC-I) helps identify families with Hereditary Non Polyposis Colorectal Cancer(HNPCC). 60% of ACI families have a hereditary defect in a mismatch repair gene, resulting in tumor microsatellite instability (MSI). Cancer risks in these HNPCC families are very high and well defined. Other AC-I families have no evidence of a mismatch repair defect (microsatellite instability low or stable, MSI-L/MSS). Risks in such families are unknown. We compared cancer risks in AC-I families with MSI versus MSI-L/MSS. **Methods:** We identified 161 AC-I pedigrees that could be assigned to Group A: due to hereditary mismatch repair defects (MSI) or to Group B: AC-I families not due to defective mismatch repair (MSI-L/MSS) as determined by tumor testing. We analyzed cancer risks compared to SEER data in the 1st and 2d degree relatives of the triad. **Results:** 3,422 relatives (excluding triad members) were included in analyses. Cancer risks in Group A (MSI) showed the expected increased risk for cancers consistent with HNPCC. Group B (MSI-L/MSS) showed only moderate increased risk for colorectal cancer. In Group A, (n=1855) relative risks(CI) were: colorectum:6.1(5.3-7.1); uterus:4.1(2.9-5.6); small bowel:7.6(2.9-16.5); small bowel:7.6(2.9-16.5); kidney/ureter: 3.1(1.9-4.8); ovary:2.0(1.0-3.5). In Group B (n=1567), colorectal RR=2.3(1.8-3.0). No other cancers were significantly increased. **Conclusion:** Families fulfilling the AC-I criteria can be divided into true HNPCC/Lynch syndrome and those that do not have true HNPCC. ACI families without DNA mismatch repair deficiency should be counseled that it is very unlikely they have HNPCC; screening recommendations can be customized, based on these data and an individual's family history. Supported by NCI, National Institutes of Health RFA #CA-95-011.

Matching patterns of genomic imbalances point to common pathways of disease progression in hereditary and non-hereditary retinoblastomas. *S. Schneider*¹, *D.R. Lohmann*², *L. Hehn*¹, *E. Lieth*¹, *S. Herzog*¹, *H. Rieder*¹. 1) Clinical Genetics, Philipps-University, Marburg, Hessen, Germany; 2) Institute of Human Genetics, University Hospital, Essen, Nordrhein-Westfalen, Germany.

Genomic imbalances have been demonstrated in 65-95% of Retinoblastomas (Rb) and are more frequent in Rb of older than in those of younger patients. Because patients with hereditary Rb are younger than patients with non-hereditary Rb, we investigated if both groups differ from each other by the patterns of copy number changes (CNC) as revealed by comparative genomic hybridization (CGH). Tumors of 100 patients with unilateral isolated non-hereditary Rb (nhRb) and of 65 patients with bilateral hereditary Rb (hRb) were studied. In all tumors biallelic RB1 inactivation was verified. Both tumor groups showed an increase of genomic imbalances with age. The fractions of abnormal tumors in patients with ages ≤ 12 months was 43.5% in hRb and 40% in nhRb. In children with ages > 12 months 73.7% of hRb and 91.4% of nhRb showed abnormal tumors. In hRb and nhRb gains of 6p (55% and 32.3% of abnormal tumors) and 1q (41% and 15.4%), as well as losses of 16/16q (37% and 9.2%) were most frequent. Moreover, gains at 1q and losses at 16/16q were restricted to patients with ages > 12 months in both groups. Retinal development and retinoblast differentiation progress from central (macula) to peripheral (ora serrata). Thus, in children of younger ages the proportions of retinoblasts and of differentiated retinal cells is different from that in older children. The observed increase of CNCs in nhRb and hRb with age may, therefore, reflect differences in the genetic pathways of tumor progression depending on different differentiation stages and/or locations of the transformed retinal cells. Funded by the DFG, Ri-1123/1-1.

Identification of genes associated with ovarian cancer metastasis using microarray expression analysis. *J.M. Lancaster¹, H.K. Dressman⁴, J.L. Pittman⁵, A.H. Henriott³, R.A. Sayer¹, J. Gray², R.S. Whitaker³, M. West⁵, J.R. Marks⁶, J.R. Nevins⁴, A. Berchuck³.* 1) Interdisciplinary Oncology, H. Lee Moffitt Cancer Center, Tampa, FL; 2) Institute of Medical Genetics, University Hospital of Wales, Cardiff, CF14 4XN, UK; 3) Departments of Obstetrics and Gynecology/Division of Gynecologic Oncology, Duke University Medical Center, Durham, NC 27710; 4) Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC 27710; 5) Howard Hughes Medical Institute, Institute for Genome Sciences and Policy, Institute of Statistics and Decision Sciences, Duke University Medical Center, Durham, NC 27710; 6) Surgery, Duke University Medical Center, Durham, NC 27710.

The transition from early to advanced stage ovarian cancer is a critical determinant of survival, but little is known about the molecular underpinnings of ovarian metastasis. We applied Affymetrix U95Av2 microarrays to characterize the molecular alterations that underlie the development of omental metastasis from 47 epithelial ovarian cancer samples collected from multiple sites in 20 patients undergoing primary surgery for advanced stage serous ovarian cancer. Fifty-six genes demonstrated differential expression between ovarian and omental samples ($p < 0.01$), and 20/56 (36%) differentially expressed genes have previously been implicated in metastasis, cell motility or cytoskeletal function. Ten of 56 genes (18%) are involved in p53 pathways. Bayesian statistical tree analysis was applied to validate the significance of the gene expression patterns, and identified 27 genes that could accurately predict tumor site (ovary vs. omentum) in 87% of cases in cross-validation studies. Nine of the 27 genes (GRP135, FHL-2, GA, LAMC2, MAGE-A10, CDK5, RPS6KB1, AIM2, SLIT3) have previously been shown to be involved in oncogenesis, and 10/27 genes (LAMC2, APOBEC2, FHL, STAR, PARD6B, ELAVL1, ANGPT1, CDK5, RPS6KB1, RFXAP) have been implicated in p53 pathways. Using genome-wide expression technology and novel statistical and gene pathway analyses we have identified a distinct genetic profile that underlies omental metastasis from epithelial ovarian cancer.

Contribution of Fluorescent In Situ Hybridization analysis to detect *BCR-ABL* complex in patients with Philadelphia negative chronic myelogenous leukemia. *M. Soto Quintana, A. Rojas-Atencio, F. Álvarez Nava, K. Urdaneta.* Unidad Genética Médica, Universidad del Zulia, Maracaibo Zulia, Venezuela.

Chronic myelogenous leukemia (CML) is genetically characterized by the presence of the Philadelphia (Ph) chromosome that arises from t(9;22)(q34;q11). About 95% of patients with CML have the translocation or a variant of this. The remaining 5% has a submicroscopic translocation that is not evidenced by conventional cytogenetics methods (Ph negative). Fluorescent In Situ Hybridization (FISH) analysis, however, reveals the t(9;22) and formation of the *BCR-ABL* fusion gene. The translocation fuses the *ABL* and *BCR* genes by inserting the *ABL* proto-oncogen located at 9q34.1 to the *BCR* region at 22q11.2. This chimerical gene codes for an abnormal protein which has tyrosine kinase activity and significant leukemogenic potential. The objective of this study was to detect of the *BCR-ABL* complex in patients with Ph negative chromosome CML. Bone marrow samples from 15 patients with CML were analyzed. To determine the *BCR-ABL* rearrangement by FISH we used the probe LSI *BCR-ABL* and hybridization protocol that were supplied by VYSIS. FISH analysis showed that all patients presented the fusion *BCR-ABL*. Our study demonstrates the significance of this assay in the detection of the *BCR-ABL* complex in patients with Philadelphia negative CML. Thus, FISH is a crucial to the accurate diagnosis and correct medical management of this patients.éé.

Statistical Analysis of cDNA Array CGH Data in Breast Cancer Cell Lines and Tumors. *S. Colby¹, S.B. Bull^{1,2}, A. Bane¹, L. Bosnoyan-Collins¹, N. Gokgoz¹, S. Minaker¹, I.L. Andrulis^{1,3}.* 1) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON; 2) Department of Public Health Sciences, University of Toronto, Toronto, ON; 3) Department of Molecular and Medical Genetics, University of Toronto, Toronto, ON.

The identification of genes involved in cancer has benefited from detection of changes that occur in tumor cells. Array-based comparative genomic hybridization (*a*-CGH) provides a measure of DNA copy number variation across the entire genome an alternative to identifying novel genetic targets by studying multi-case families using linkage methods. We have completed *a*-CGH on 5 breast cancer cell lines (BT474, MDA-MB231, SKBR3, T47D and UACC) and on over 50 tumor DNAs from a cohort of axillary node-negative cases from the Toronto area. In the first stage of the analysis, we delineated regions of chromosomal loss or gain for each patient using the Bioconductor package DNACopy (Olshen and Venkatraman, 2004). Each region was assigned an alteration type (i.e., normal, loss/deletion, gain/amplification) based on the log copy number ratios of the clones within the region. In the second stage of the analysis, the alterations were tabulated yielding a ranking according to alteration frequency of the top regions for further examination.

Analysis of the BT474 cell line detected known amplifications on 17q11.2-22 and 20q12-13.3. We detected gains in 80% of the tumors, losses in 47% of the tumors, and found no detectable alteration in only 5 tumors. Many of the the regions of alteration detected in the tumors have been implicated in breast cancer through conventional CGH.

Gonadal tumors at an early age in patients with mixed gonadal dysgenesis. *I. Palma¹, Y.R. Pena², K. Nieto¹, R. Alvarez¹, L. Erana², L.M. Dorantes², S. Kofman-Alfaro¹, G. Queipo¹.* 1) Dept Genetics, Hosp General de Mexico, Mexico City, Mexico; 2) Intersex Clinic. Hospital Infantil de Mexico-Federico Gomez, Mexico City, Mexico.

Gonadoblastoma is an unusual mixed germ cell-sex cord stromal tumor that occurs mostly in individuals with abnormal gonadal development and a karyotype with Y chromosome or Y-derived sequences. Half of the gonadoblastomas show germ cell overgrowth leading to a typical dysgerminoma transformation. The age at diagnosis is variable but they are very rare at early ages. We present 5 patients with different types of gonadal dysgenesis, in 4 of them (cases 1-4) the tumor was recognized in early childhood while in case 5 a dysgerminoma was observed at age 15. In these patients we found different types of tumors: juvenile granulosa cell tumor beside a gonadoblastoma, bilateral gonadoblastoma and dysgerminoma. FISH analyses performed to evaluate the X and Y cell distribution between the tumor and the dysgenetic gonadal tissue, showed that Y cells were more frequent in the tumor than in the gonad. We discuss these findings and the genesis of these tumors in young children and recommend early surgical management of the dysgenetic gonads.

Urovysion assay on fresh urine samples: needs to find a general consensus. *D. Bettio*¹, *A. Venci*¹, *M. Corinti*², *P. Graziotti*². 1) Cytogenetic Laboratory, Humanitas Clinical Institute, Rozzano, Milan, Italy; 2) Urology Operative Unit, Humanitas Clinical Institute, Rozzano, Milan, Italy.

The study was performed in order to introduce FISH on urine as a powerful tool for the detection, follow-up and prediction of bladder tumors. Some interesting observations led us to correlate the FISH results with the tumors' biological behaviour. FISH was performed on fresh urine from 35 patients without prior knowledge of biopsy, cystoscopy and cytology results. Patients were selected following several criteria: all except 3, presenting with hematuria, had a history of transitional cell carcinoma. We used the two more common criteria for FISH positivity: one recommended by the manufacturer (Vysis) that we call maximum criteria (MC) and the other by Bubendorf et al. (2001). The latter sets the cutoff for positivity at more than 2 cells with an abnormal signal copy number of at least 1 of the 4 probes, that we call minimum criteria (mc). In 13 MC+ cases the presence of the tumor was confirmed, while the 11 negative ones had TUR and/or therapy before FISH was performed. Of the 3 cases with hematuria, 2 were MC+ and 1 mc+. Twelve out of 35 cases turned out to be MC- but mc+. So far, after 1 year average follow-up, 4 turned MC+ and 1 remained MC-. Three MC+ patients had tumor recurrence within 6 to 18 months following the positive FISH result. The predictivity of FISH led us to choose a strict follow-up in some patients. So far we have 9 false positive cases with several MC+ results without evidence of the disease. Since we defined a negative at 24-month follow-up we consider these cases still in progress. In 3 MC+ and 2 mc+ cases we observed 9p21 deletion associated with monosomy 17 as the sole anomalies. Because of this finding we screened the 35 cases looking for monosomy 17 in at least in 1 cell. For 6 out of the 20 found we had a follow-up after at least 6 months demonstrating an increased number of aneuploidy cells and so far 3 patients had tumor recurrence. This observation indicates that monosomy 17 might be the second genetic alteration after loss of P16 and is suggestive of recurrence risk. Based on this result we suggest that monosomy 17 should be included in the criteria for positivity.

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The effects of trichostatin A on adenovirus-mediated p16 gene transfer in Balb/c nude mouse. *M. Park, O. Kim, S. Lim, J. Kim, C. Song, H. Jung, H. Kang.* Toxicological Research Dept., NITR, KFDA, Seoul, Korea.

To enhance therapeutic effects of gene therapy, the combinational strategy with chemotherapeutic agent is rapidly increased for cancer treatment. But there is limited information about efficacy and safety of combinational gene therapy. We investigated the effects of trichostatin A, a antitumor agent, on Ad5CMV-p16 (p16-expressing adenoviral vector) in non-small cell lung cancer. We confirmed that replication competent adenovirus and contaminations of microorganism were not detected in our adenoviral vectors (Ad5CMV-p16, Ad5CMV-LacZ) by PCR and mycoplasma test. Trichostatin A increased the inhibition effect of Ad5CMV-p16 on A549 cell-induced lung tumor formation and A549 xenograft tumor growth in Balb/c nude mice. As a safety concern, we investigated biodistribution changes after combinational treatment of Ad5CMV-LacZ and trichostatin A. We found that -galactosidase activities were detected in liver, kidney, spleen, and lung after tail vein injection of Ad5CMV-LacZ. Trichostatin A enhanced the transduction of Ad5CMV-LacZ into lung and changed biodistribution pattern in kidney. Taken together, we suggest that combinational gene therapy with trichostatin A could enhance the transfection efficiency of Ad5CMV-p16, and it will be helpful to reduce the titer of adenoviral vector. Further studies will be needed to investigate whether CAR and integrin expression would be changed or not by trichostatin A in vivo.

Multiplex PCR / Liquid Chromatography assay for detection of gene rearrangements: Application to the RB1 gene. *C. Dehainault¹, A. Laugé¹, V. Caux Moncoutier¹, S. Pagès Berhouet¹, F. Doz², L. Desjardins³, J. Couturier^{1, 4}, M. Gauthier Villars¹, D. Stoppa Lyonnet^{1, 4}, C. Houdayer¹.* 1) Genetics; 2) Pediatric Oncology; 3) Ophthalmology; 4) Inserm U509, Institut Curie, Paris, France.

Screening for large gene rearrangements is established as an important part of molecular medicine but is also challenging. A variety of robust methods can detect whole-gene deletions but will fail to detect more subtle rearrangements that may involve a single exon. Here we describe a new versatile and robust method to assess exon copy number, termed Multiplex PCR/Liquid Chromatography assay (MP/LC). Multiple exons are amplified using unlabelled primers, then separated by ion-pair reversed-phase high performance liquid chromatography, and quantitated by fluorescent detection using a post-column intercalation dye. The relative peak intensities for each target directly reflect exon copy number. This novel technique was used to screen a panel of 121 unrelated retinoblastoma patients, previously tested using a reference strategy i.e. a combination of FISH, QMPSF and RT PCR analyses. MP/LC correctly scored all deletions and evidenced a previously undetected RB1 duplication, the first to be described. MP/LC appears as an easy, versatile, and cost-effective method, which is particularly relevant to DHPLC users since it broadens the spectrum of available applications on a DHPLC system.

A Tiling Resolution Bacterial Artificial Chromosome Array Delineates Distinct Regions of Genetic Alteration in Breast Cancer Genomes. *T.P.H. Buys, A. Shadoo, S. Yip, K.A. Gelmon, T.O. Nielsen, W.L. Lam.* British Columbia Cancer Agency, Vancouver, BC, Canada.

Breast cancer is the most commonly diagnosed malignancy in Canadian women. Genetic alterations that drive disease progression may differ between disease subtypes. The newly developed whole genome Sub-Megabase Resolution Tiling-set (SMRT) array has moved genomic profiling beyond marker-based analysis, comprehensively identifying copy number alterations. We have identified known and novel recurrent genetic alterations by comparing whole genome profiles of ten cases of the postulated basal subtype of disease, which has the ER-, Her2-, KRT5/6+, PR-immunohistochemical (IHC) staining pattern. These cases were identified from archived breast cancer samples at the Vancouver General Hospital. DNA was extracted from microdissected malignant cells for SMRT array comparative genomic hybridization (CGH) analysis. Genome profile comparison revealed multiple recurrent gains and losses of DNA segments. Amongst the numerous alterations identified, recurrent novel alterations were found, including copy number gains at 1p32.1-p32.2, 5p15.2, 6p22.3, 10p14 and losses at 8p11.2 and at multiple segments between 19q13.2-q13.3. Array-based results will be verified by fluorescence in situ hybridization analysis. This work represents the highest resolution analysis of breast cancer genomes to date. SMRT array CGH profiles revealed multiple recurrent genetic alterations, including sub-megabase regions which harbor candidate tumor suppressors or oncogenes potentially crucial to the development of BC.

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High resolution mapping of genomic imbalance and gene expression profiles associated with differential chemotherapy response in serous epithelial ovarian cancer. *M. Bernadini¹, P. Shaw¹, C-H. Lee¹, B. Beheshti¹, H. Bagley¹, M. Prasad¹, S. Minkin¹, J. Murphy¹, B. Rosen¹, W. Lam², S. Watson², P. Macgregor¹, J.A. Squire¹.* 1) University Health Network, Toronto, Ontario, Canada; 2) British Columbia Cancer Agency, Vancouver, British Columbia, Canada.

This study is applying whole genome analytical methods at the DNA and RNA levels in serous epithelial ovarian cancer (SEOC) in the context of clinical parameters such as tumor stage, CA125 levels, treatment response and survival. In the first phase of this study high- resolution microarray comparative genomic hybridization (CGH) was used to identify genomic regions associated with chemotherapy resistance in epithelial ovarian cancer. The analysis has confirmed the presence of copy number alterations at specific chromosomal regions in 26 tumors. Gains at 1q, 3q, 8q, 12p and 20q as well as losses at 1p, 4q, 6q, 8p, 9q, 13q, 16q, 17p, 18q, were consistent with previously reported recurrent genomic imbalance detected by metaphase CGH. A systematic bioinformatics analysis using the high resolution CGH array datasets has further allowed us to identify small sub-genomic regions that discriminate drug resistance as determined by the kinetics of serum CA125 levels following chemotherapy. These regions include recurrent losses at 1p36.33 (2.2Mb) and 6q24.3-25.2 (18.4Mb) with gains being detected at 1q42-44 (25.6Mb) and 13q12.2-13.1 (6.3Mb). Expression profiles were obtained in a companion parallel study and findings concerning novel pathways previously implicated in cis-platinum adduct repair will be reported. The information gained in this study provides the first step to identifying discriminating regions of genomic imbalance that may potentially play a role in predetermining patients for specific chemotherapeutic regimens.

RNA Expression Changes and Concordance with DNA Copy Number Alterations in Serous Epithelial Ovarian Carcinoma Associated with Resistance to Chemotherapy. *C-H. Lee¹, M. Prasad¹, M. Bernadini^{2,3}, B. Beheshti², P. Marrano², H. Begley³, P. Shaw³, A. Covens⁵, J. Murphy⁴, B. Rosen⁴, S. Minkin², J.A. Squire^{2,3}, P.F. Macgregor¹.* 1) Microarray Centre, Clinical Genomics Center, University Health Network, Toronto, Ontario, Canada; 2) Ontario Cancer Institute, Princess Margaret Hospital, University Health Network, Toronto, Ontario, Canada; 3) Department of Laboratory Medicine and Pathobiology, University of Toronto, Ontario, Canada; 4) Department of Gynecological Oncology, University of Toronto, Ontario, Canada; 5) Toronto Regional Cancer Centre, Sunnybrook and Women's College Hospital, Toronto, Ontario, Canada.

To improve our knowledge of resistance to chemotherapy in Serous Epithelial Ovarian Cancer (SEOC) patients, we used RNA profiling by cDNA microarrays on a cohort of tumors obtained from patients sensitive and resistant to carboplatin/taxol treatment. Based on their CA 125 levels before, during, and after treatment, ten SEOC samples were selected from patients who exemplified extreme cases of differential response to the drug regimen. We identified RNA signatures distinct to ovarian tumors resistant or sensitive to chemotherapy. Within these signatures five genes (GAPD, HMGB2, HSP70, GRP58 and HMGB1), previously shown to form a nuclear complex associated with resistance to DNA-conformation altering chemotherapeutic drugs in in vitro systems, may represent a novel class of genes associated with in vivo drug response in ovarian cancer patients. A concordance analysis of overall genomic imbalance (presented by Squire et al) and RNA expression change indicated only weak DNA copy number dependence. Thus, the goals of this study were to identify RNA profiles that are predictive of treatment failure and to determine whether gene copy number differences are reflected at the RNA level.

P16 hypermethylation, a possible epigenetic risk factor in a familial esophageal squamous cell carcinoma as compared to CEA tumor marker. *H.R. Raziee^{1,2}, S. Afsharnezhad¹, H. Khallaghi¹, K. Ghaffarzagdegan³, M.R. ghavamnasiri², M.R. Abbaszadegan¹.* 1) Division of Human Genetics, Immunology Research Center, Bu- Ali Research Institute, Mashhad University of Medical Science (MUMS); 2) Department of Radiation Oncology, Omid Cancer Hospital, MUMS; 3) Department of Pathology, Omid Cancer Hospital, MUMS, Mashhad, Iran.

New tumor markers for early detection in esophageal squamous cell carcinoma (ESCC) may improve the life expectancy for this disease. Methylation of P16 may be detected in serum DNA of cancer patients as a tumor marker. DNA from serum and blood of ESCC patients for p16 hypermethylation in Northeastern Iran showed a family with clustering of ESCC. Three histological proven cases of ESCC in two consecutive generation and other deceased cases with history of ESCC in this pedigree provoked a p16 hypermethylation study. DNA from blood of 28 family members in 3 generations and 30 healthy volunteers was examined for methylation status of p16 promoter using methylation specific PCR (MSP). The results revealed aberrant p16 promoter methylation in blood from 64.3% (n=28) of ESCC family members and none 0% (n=30) in normal volunteers. The proband with ESCC had p16 hypermethylation in DNA extracted from tumor, blood and serum. Seventeen asymptomatic family members also had p16 hypermethylation in their blood. Five of 28 family members had negative endoscopy results for ESCC, but 4 of these members had p16 hypermethylation in their blood. The family members with negative endoscopy but positive p16 promoter methylation are being followed for any signs of ESCC development using chromoendoscopies. In sporadic ESCC in northeastern Iran, 73.3% (n=30) of tumor tissue samples had p16 hypermethylation. Serum and blood samples from these patients showed p16 hypermethylation in 26.6% and 43.3% respectively. Results were compared with the serum Carcinoembryonic antigen (CEA), a tumor marker with elevations in tumors of gastrointestinal tract. Only one serum sample presented elevated CEA. Results indicated that aberrant p16 methylation may be a valuable epigenetic tumor marker for identification of individuals in high risk ESCC families.

GDNF INDEPENDENT ACTIVATION OF A Src/JAK/STAT3 PATHWAY BY RET-FMTC MUTANTS Y791F

AND S891A. *R.M.W. Hofstra¹, I. Plaza Menacho¹, R. Koster¹, A. van der Sloot², W.J. Quax², T. van der Sluis³, H. Hollema³, O. Gimm⁴, G. Burzynski¹, C.H.C.M. Buys¹, B.J.L. Eggen⁵.* 1) Dept. Medical Genetics, Antonius Desinglaan 4, 9713 AW Groningen University of Groningen, Groningen, Netherlands. R.M.W.Hofstra@medgen.azg.nl; 2) Dept. Pharmaceutical Biology, Univ Groningen Groningen, The Netherlands; 3) Dept. Phatholgy, Univ Hospital Groningen, Groningen, The Netherlands; 4) Dept. Surgery, Martin-Luther-Universitat Halle-Wittenberg, Germany; 5) Dept. Developmental Genetics, Univ Groningen, Groningen, Netherlands.

The RET proto-oncogene encodes a receptor tyrosine kinase whose dysfunction plays a crucial role in the development of several neural crest disorders. Distinct activating RET mutations are found associated with Multiple Endocrine Neoplasia types 2A (MEN2A), 2B (MEN2B) and Familial Medullary Thyroid Carcinoma (FMTC). Despite clear correlations between the mutations found in these cancer syndromes and their phenotypes, the molecular mechanisms connecting the mutant receptor to the different disease phenotypes are far from completely understood. Luciferase reporter assays in combination with immunoprecipitations, western and immunohistochemical analyses were performed in order to characterize the signaling properties of two FMTC-associated RET mutations, Y791F and S891A, respectively, both affecting the tyrosine kinase domain of the receptor. We show that these FMTC mutations lead to dominantly activating monomeric receptors which are autophosphorylated and activated independently of GDNF. Moreover, we demonstrate that the dysfunctional signaling properties of these mutations, when compared to wild type RET, involve the constitutive activation of STAT3. This STAT3 activation is mediated by a signaling pathway involving Src and JAK1-2. Three-dimensional modeling of the RET catalytic domain suggests that the structural changes promoted by the respective amino acid substitutions lead to a more accessible substrate binding conformation. The in vitro data are supported by the results of immunohistochemical analysis of FMTC tumor samples support that show nuclear localization of Y705-phosphorylated STAT3 as well as a high degree of RET expression at the plasma membrane.

Identification of tumor associated SNPs based on EST analysis. *W. Ding¹, M. Kostich¹, P. Qiu¹, L.Q. Wang², J.R. Greene¹, M. Hernandez¹.* 1) Bioinformatics Group, Discovery Technology Department, Schering-Plough Research Institute, Kenilworth, New Jersey 07033; 2) GenScript Corporate, 120 Centennial Avenue, Piscataway, New Jersey 08854.

Carcinogenesis occurs, at least in part, due to the accumulation of mutations in critical genes that control the mechanisms of cell proliferation, differentiation and death. Expressed Sequence Tags (ESTs) are derived from cDNA libraries generated from a vast number of normal and disease tissues. By statistical analysis of human ESTs, we detected 176,207 candidate Single Nucleotide Polymorphisms (SNPs). We manually curated and catalogued EST cDNA tissue libraries into non-tumor libraries and tumor libraries and examined the association between individual SNPs and tumor tissues. A total of 5152 SNPs were identified which were present at higher allele frequencies in tumor compared to normal tissues. A subset of 1955 (37.9%) SNPs induce amino acid changes to the protein coding sequences. This approach identified many SNPs which have been previously associated with carcinogenesis, as well as a number of SNPs that now warrant further investigation. This genome-wide in silico approach can assist in detection of tumor associated SNPs and help to elucidate the genetic mechanisms underlying the development of cancer.

UMD-BRCA1 and BRCA2 mutation databases. *D. Hamroun*^{1, 2}, *S. Bezieau*², *I. Biché*³, *B. Bressac-de Paillerets*³, *F. Coulet*³, *M.C. Gorisse*³, *A. Hardouin*³, *M. Longy*³, *S. Olschwang*³, *J.P. Peyrat*³, *C. Philippe*³, *P. Rostagno*³, *T. Noguchi*³, *O. Sinilnikova-Evrard*³, *D. Stoppa Lyonnet*³, *C. Toulas*³, *N. Uhrhammer*³, *M. Claustres*², *C. Beroud*², *R. Lidereau*¹. 1) Rene Huguenin Center, Saint Cloud, France; 2) Dept of Molecular Genetics, CHU de Montpellier, Montpellier, France; 3) Sub group of laboratories of cancer genetic's group of FNCLCC (GGC).

BRCA1 and BRCA2 are the major genes responsible for ovary and/or breast cancers. Germline mutations are mainly small size mutations and are distributed throughout both genes. If the deleterious character of truncating mutations is generally assumed, only few data are available for missense mutations biological significance. The development of databases collecting mutations of both BRCA1 and BRCA2 genes characterized from French families was performed with the UMD "Universal Mutation Database" generic software. In addition to the full molecular description of each mutation (consequence at the DNA, RNA and protein levels), we collected clinical information from genetic counseling of the GGC.

So far we have collected data from 1207 families for BRCA1 gene and 842 families for BRCA2 gene among those 736 and 377 families respectively have deleterious mutations. Among the BRCA1 deleterious mutations, 48.9% are out of frame deletions, 0.8% in frame deletions, 18.2% out of frame insertions, 6% missense mutations, 22.1% nonsense mutations and 11.6% intronic mutations. For BRCA2 64.7% are out of frame deletions, 10.9% out of frame insertions, 0.3% missense mutations, 21.2% nonsense mutations and 2.9% intronic mutations.

Mutations of either gene can be found in various familial situations (numbers are given for BRCA1 and BRCA2, respectively): breast cancer (43%; 63%); breast and ovary cancers (47%; 25%); ovary cancer (5%; 4%); young isolated breast or ovary cancer (4%; 3%); man breast cancer (0%; 5%); other (2%; 1%).

The computation of these data should help in the identification of deleterious mutations among variations of unknown biological significance. This should prove particularly useful for families with missense mutations to better design patients follow-up.

Bone marrow engraftment chimerism monitoring by short tandem repeat (STR) analysis. *L.M. Hire, P. Labrousse, J. Garces, B.A. Allitto.* Genzyme Genetics, Westborough, MA.

Objective: Monitoring of hematopoietic cell chimerism is important for establishing graft rejection and disease relapse. We evaluated a method for monitoring chimerism using fluorescent multiplex PCR of STRs and compared the limit of detection and heterogeneity of various STR loci available in the AmpFLSTR kits from Applied Biosystems. **Methods:** Blood and bone marrow mixtures were made based on cell counts or spectrophotometric DNA measurements. Straight and mixed samples were subjected to multiplex PCR using the ProFiler Plus and COfiler kits followed by capillary electrophoresis with fluorescent detection. Percent chimerism was calculated as a ratio of allele peak heights measured in RFUs between donor (D) and recipient (R) alleles using the formula: $[(R1+R2)/(R1+R2+D1+D2)] \times 100$. **Results:** The sensitivity of allele detection and the total number of informative alleles were comparable between pre and post extraction mixtures for both blood and bone marrow specimens. A 5% detection limit was consistently measured for a subset of the markers tested, although 1% detection was observed for a number of allelic combinations. Preliminary data suggest that on average 1 in 4 markers should be detected as informative in 5% mixtures using these STR panels. To ensure accurate calculation of percent chimerism, samples should be analyzed in duplicate with averaging of peak intensities. **Conclusions:** The ratio of allele peak heights following PCR amplification and fluorescent detection of STR markers serves as an adequate and reproducible semi-quantitative measure of the relative amounts of both the major and minor component in cell mixtures such as those observed following bone marrow engraftments. We developed several criteria around RFUs and genotype combinations to ensure detection of at least 5% chimerism in a clinical assay. While both the ProFiler Plus and COfiler panels are adequate for chimerism detection, the COfiler panel produced more informative markers on average at a 5% detection limit than the ProFiler Plus panel. In our sample set, D8S1179, D16S539, and D3S1358 were the most informative markers in mixtures where the minor component was present at 5%.

High-Risk Breast Cancer and a 5'UTR Triplet Repeat Polymorphism in the Glutamate-Cysteine Ligase Catalytic Subunit (GLCLC) Gene. *A.R. Kallianpur^{1,4,5}, A.S. Willis^{3,4}, B.W. Christman^{1,4,5}, M.L. Summar^{2,3,4}.* 1) Departments of Medicine; 2) Pediatrics; 3) Molecular Physiology & Biophysics; 4) Vanderbilt University Medical Center, Nashville, TN, USA; 5) TN Valley Health Services VA Medical Center, Nashville, TN, USA.

Oxidant stress, the increased production of free radicals that damage cellular lipids, proteins and DNA, is implicated in the pathogenesis of many diseases, including cancer. The tripeptide glutathione is an important scavenger of free radicals in vivo, and the enzyme glutamate L-cysteine ligase (GLCL) catalyzes the first and rate-limiting step in glutathione synthesis. We investigated polymorphisms of a previously described GAG trinucleotide repeat in the 5'UTR of the gene (GLCLC) that encodes the catalytic subunit of this enzyme in 132 patients undergoing bone marrow transplantation for high-risk cancers. The mean age of this transplant cohort was 43.710.6 years, gender composition was 60% female, and the underlying diagnosis was breast cancer in 35 women and hematological cancer in 97 patients. Patients with varying GAG repeat alleles were similar with respect to age, race, and gender. The observed GAG repeat allele frequencies in this cohort were also not significantly different from frequencies previously described by our group in a population representative of middle Tennessee. However, the frequency of the GLCLC GAG 8/9 genotype in breast cancers was higher (14.3%) than frequencies in all other cancer patients in this cohort (1.0%, $P=0.005$), women without breast cancer (0%, Fisher's exact $P=0.014$), and in the general TN population (6.5%, $P=ns$). This strong association with breast cancer was unchanged by adjustment for age and the hemochromatosis C282Y mutation (also on chromosome 6p), previously associated with breast cancer in this cohort (adjusted OR 14.1, $P=0.023$). Our findings in this cancer cohort suggest that polymorphisms in the 5'UTR GAG repeat of GLCLC may be important determinants of the clinical behavior of breast cancer in vivo and may play a role in the pathogenesis of this disease. Studies of GLCLC GAG polymorphisms in larger numbers of women with breast cancer and in normal controls are needed.

Cellular responses to genotoxic stress induced by temozolomide in an astrocytic cell line (U343 MG-a). *M.O. Bahia*^{1,2}, *C.L. Bassi*¹, *S.S. Mello*¹, *R.S. Cardoso*¹, *A.L. Fachin*¹, *G.A.S. Passos*⁵, *E.A. Donadi*³, *C.G. Carlotti Jr*⁴, *E.T. Sakamoto-Hojo*^{1,6}. 1) Depto. de Genética, FMRP, USP, SP, Brazil; 2) Depto. de Patologia, UFPA, PA, Brazil; 3) Clínica Médica, FMRP, USP, SP, Brazil; 4) Depto. de Cirurgia e Anatomia do Hospital das Clínicas, FMRP, USP, SP, Brazil; 5) Faculdade de Odontologia de Ribeirão Preto, USP, SP, Brazil; 6) Depto. de Biologia, FFCLRP, USP, SP, Brazil.

Alkylating agents, such as temozolomide (TMZ), have been widely included in the chemotherapeutic regimens for patients with gliomas. However, there is still a relative lack of understanding of the action of TMZ in glioma cells. In the present study, we analyzed the cellular responses to TMZ, in terms of clonogenic efficiency, alterations in cell cycle kinetics and apoptosis in an astrocytoma cell line (U343MG-a). In addition, the profiles of gene expression were studied by cDNA microarrays. Clonogenic efficiency was also assessed in an U343MG-a cell line transfected with a tetracycline repressor system harboring the p16 gene. Cell treatment with TMZ concentrations ranging from 10 to 360M induced a significant reduction in the reproductive capacity of U343 MG-a cells. However, an increased resistance to TMZ was observed in the transfected cell line after induction of the p16 gene. Cell cycle kinetics changes were also observed after treatment with 5 and 10M TMZ, while the induction of apoptosis was not evident until 72h after drug treatment. The analysis of gene expression by cDNA microarrays indicated many up-regulated genes observed at 48h after TMZ (10M) treatment. Among them, many genes known to participate in MAPK/JNK/c-jun pathways and genes controlling the cell cycle. Genes participating in DNA repair processes (BER, NHEJ and HRR) were also activated, indicating the signaling response mechanism towards the base-alkylation caused by TMZ, as well as against the secondary-damage, both of them being susceptible to the DNA repair machinery. Further experiments are under course with the trasfected U343 MG-a cell line in order to evaluate the effects of TMZ treatment after induction of the p16 gene on the cell cycle kinetics, apoptosis and gene expression.

Localization and protein interaction of Tsga10 and developmental changes of the gene/protein expression in mice embryo, testis, brain and some malignancies in relation to cellular division and brain development. *B. Behnam*¹, *MH. Modarressi*², *S. Manistre*¹, *S. Povey*¹, *F. Van der Hoorn*², *J. Wolfe*¹. 1) Dept Biol/Galton Lab, University College London, London, NW1 2HE United Kingdom; 2) Department of Biochemistry & Molecular Biology, University of Calgary, Canada T2N 4N1.

Tsga10-encoded protein contains a putative myosin/Ezrin/radixin/moesin (ERM) domain and has a 27-KD fibrous sheath component of sperm tail after modification. High level of TSGA10 expression is reported in some cancers. In this study we attempted to determine putative functions for Tsga10 during every active dividing cell process including spermatogenesis, neurogenesis, embryogenesis and carcinogenesis. To address this, we analysed the pattern of expression and localisation of this protein in mouse testicular cells during postnatal development and adulthood, in developmental stages of mouse embryos, in adult mice brain and in two types of malignancies including human germ cell tumour and acute myeloid leukemia. Also a testis cDNA library is screened to study Tsga10 protein interaction to other proteins by yeast two hybrid assays. Using Northern blotting, in situ hybridisation and immunostaining of testis sections we showed Tsga10 mRNA and Tsga10 protein expression in spermatogonia at pachytene to mature elongated sperms. We also found that Tsga10 localises around the nucleus, which indicates a potential porin activity of this protein. By RT-PCR and immunoreactivity study, we show Tsga10 expression mouse embryogenesis as well as brain development, germ cell tumour and acute promyelocytic leukemia. This pattern of Tsga10 gene/protein expression suggests that Tsga10 involved in spermatogenesis, neurogenesis, differentiation, tumorigenesis and embryo development. It is highly expressed with two transcripts in testis and whole embryo but with only one transcript in brain embryo. Three proteins are found as interacting proteins with Tsga10 in sperm tail. We localized a novel one among them in principal piece of sperm tail exactly where Tsga10 FS protein was localized. We hypothesize a scenario in spermatozoa, axonal transport in the brain and synaptogenesis and all actively dividing cells for Tsga10 as a structural protein.

GERMLINE AND SOMATIC MUTATIONAL ANALYSIS, GENOTYPE/ PHENOTYPE IMPLICATIONS IN A COHORT OF 46 UNRELATED TUBEROUS SCLEROSIS COMPLEX (TSC) PATIENTS. *R. Giugliani¹, A. Puga^{1, 2}, M. Logrip², R. Beauchamp², N. Smith², M. Bowser², M. McQueen², V. Ramesh².* 1) Medical Genetics Serv, Hosp Clin P Alegre, Porto Alegre, RS, Brazil; 2) Molecular Neurogenetics Unit, Department of Neurology, Massachusetts General Hospital- East, Harvard University, Charlestown, MA, USA.

Introduction: Tuberous Sclerosis Complex (TSC), an autosomal dominant disease caused by mutations in either TSC1 or TSC2 tumor suppressor genes, is characterized by development of hamartomas, preferentially in kidneys, heart and in the central nervous system (CNS). There are various cutaneous manifestations, including multiple hypomelanotic macules, facial angiofibromas and periungual fibromas. TSC occurs in both familial and sporadic patterns. Among the familial cases, causative mutations in TSC1 and TSC2 are equally distributed. In sporadic cases, however, TSC2 can account for up to 2/3 of the diagnosed patients. Review of specific literature reveals an ongoing controversy regarding causative gene and its association with phenotypic severity in TSC, with a suggestion of TSC2 patients being more severely affected, presenting higher frequencies of seizures, mental retardation and autism. **Methods:** We screened 46 patients clinically diagnosed with TSC, using SSCP, direct sequencing, and long range PCR in order to identify their germline mutations. Additionally, five cortical tubers were analyzed via LOH and long range PCR in an effort to identify their second hit mutation (somatic mutation). **Results:** We found a statistically significant under-representation of TSC1 germline mutations among sporadic cases of Tuberous Sclerosis Complex ($P= 0,03$; Fishers exact test), but an equivalent distribution of TSC1 and TSC2 in familial cases ($P>0,05$). There were no statistically significant differences between phenotypes developed by patients with TSC1 or TSC2 mutations. **Conclusions:** In contrast to kidney angiomyolipomas and cardiac rhabdomyomas caused by TSC, the five cortical tubers analyzed here showed no loss of heterozygosity (LOH), for a panel of 8 microsatellite markers scanning TSC1 and TSC2, supporting the hypothesis of haplo-insufficiency being enough for TSC lesion development in the CNS.

Gene trapping and cDNA microarray coupled analyses of a p21-Ras mouse model for astrocytomas. *V. Lee¹, A. Guha^{1,2}, D. Kamnasaran^{1,2}*. 1) Surgery (Pediatric Neurosurgery), Hospital For Sick Children, Toronto, Ontario, Canada; 2) Surgery (Neurosurgery), Western Hospital, University of Toronto, Toronto, Ontario, Canada.

We have developed an astrocytoma mouse model with GFAP regulated expression of 12V-Ha-Ras, using embryonic stem cell transgenesis. In one line (RasD7), death occurred within ~3-4 weeks, another line (RasB8) line developed multi-focal low & high-grade astrocytomas with death over a period of ~3-6 months. The RasB8 mice are not born with astrocytomas, but progressively develop increased number of them and of higher grade. We therefore hypothesize that embryonic activated Ras creates genomic instability in the astrocytes, inducing genetic alterations leading to transformation. Taking advantage of this susceptibility, we are undertaking random mutagenesis by Gene Trapping and cDNA microarray analysis to identify early onset genes that contribute to astrocytoma development and progression in this mouse model. Astrocyte cultures were developed from P0-RasB8 (prior to development of astrocytomas), 3 months-RasB8 (harboring low- & high-grade astrocytomas), 2 week-RasD7 (harboring multiple high-grade astrocytomas) and wild-type (wt) mice. The P0-RasB8 and P0-wt astrocytes did not grow colonies in soft-agar assays, while 5-10% of the 3 months-RasB8 and > 90% of 2 week-RasD7 astrocytes did. Retroviruses were engineered encoding a Splice-acceptor type of gene trap vector, to infect ~1.2 million of the P0-RasB8 and P0-wt astrocytes, at an MOI of 1.5. Of the gene-trapped P0-RasB8 astrocytes, 0.002% became transformed as per growth in soft-agar, compared to none of the retroviral infected P0-wt astrocytes. This is consistent with our hypothesis that early expression of activated Ras makes astrocytes susceptible to transformation. We have used the Retroviral gene trap tag to identify a novel tumor suppressor genes thus far. Furthermore, to investigate additional susceptibility genes, cDNA microarray expression experiments on 15,000 murine genes were undertaken on all of the astrocyte lines. Several genes were found to be differentially expressed, results of which will be discussed in detail.

Comprehensive *NF1* screening on the selected tumorigenic cell population of dermal neurofibromas: towards an extensive study of the somatic mutation spectrum in neurofibromatosis type 1. *O. Maertens*¹, *H. Brems*², *S. De Schepper*³, *E. Legius*², *F. Speleman*¹, *L. Messiaen*^{1, 4}. 1) Center for Medical Genetics, Ghent University, Ghent, Belgium; 2) Center of Human Genetics, KULeuven, Louvain, Belgium; 3) Department of Dermatology, Ghent University, Ghent, Belgium; 4) Department of Genetics, University of Alabama at Birmingham, Birmingham, USA.

A typical hallmark of neurofibromatosis type 1 (NF1), a common autosomal dominant disorder caused by mutations in the *NF1* gene, is the occurrence of benign peripheral nerve sheath tumors or neurofibromas. These tumors comprise a mixture of cell types and the somatic *NF1* inactivation appears to occur in a subpopulation of the Schwann cells. This implicates that the somatic mutational spectrum can only be established without bias when pure cell populations harbouring the somatic mutation are analyzed. This prerequisite together with the large size of the *NF1* gene has thusfar hampered thorough investigations of the spectrum of somatic mutations in neurofibromas. The study of the somatic mutational spectrum in NF1 may provide important clues about different aspects of tumorigenesis in the disease. In order to obtain further insight into these mechanisms, we developed an improved strategy for somatic mutation detection and performed extensive *NF1* mutation screening specifically on the tumorigenic cell population of dermal neurofibromas. We detected 21 somatic mutations in 26 tumors derived from 5 NF1 patients. Of these 21 somatic mutations, 19 represented subtle mutations of different types (9 small deletions (1-19bp), 5 nonsense and 5 splicing mutations), whereas only 2 samples showed loss of heterozygosity. In conclusion, our results represent the highest somatic mutation detection rate (81%) described so far in neurofibromas illustrating that this sensitive mutation detection technique is an important tool to screen for somatic mutations in neurofibromas. Our data together with additional analysis of an extended series of tumors might help elucidate the molecular basis underlying tumorigenesis which is a major challenge in NF1 research as this might facilitate patient risk prediction and open new venues for therapeutic interventions.

BRAIN LESIONS OF TUBEROUS SCLEROSIS COMPLEX (TSC) RETAIN EXPRESSION OF HAMARTIN

AND TUBERIN. A. Puga^{1, 2}, A. Stemmer-Rachamimov³, J. Roy³, N. Smith², S. Yeul Han², V. Ramesh², R. Giugliani¹.

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Introduction: Tuberous Sclerosis Complex (TSC) a degenerative neurocutaneous disease, of autosomal dominant inheritance, affecting 1:6000 births, caused by mutation in one of two tumor suppressor genes, TSC1 or TSC2; resulting in the development of hamartomas and low-grade neoplasms in multiple organs, such as brain, heart, kidneys, and skin. **Methods:** We used specific antibodies against hamartin (HF6) and tuberin (TDSF), the gene products of TSC1 and TSC2, respectively, to analyze protein expression in kidney angiomyolipomas (AMLs), cardiac rhabdomyomas, cortical tubers, and subependymal giant-cell astrocytoma (SEGA). Samples were obtained through autopsy or surgery, from six unrelated TSC patients, with ages between 33 weeks of gestation and 61 years old, and equivalent tissues from normal controls. **Results:** Hamartin and tuberin co-localized on the cytoplasm of normal control cells. Cardiac rhabdomyomas and kidney angiomyolipomas were immunonegative for both proteins. Dymorphic neurons and balloon-cells, in cortical tubers and SEGA, were strongly immunopositive for both hamartin and tuberin, corroborating previous findings of Tuberous Sclerosis Complex CNS lesions behaving distinctly from lesions in other organs. The new polyclonal anti-tuberin antibody (TDSF), proved to be as specific as the standard polyclonal antibody (C20), in recognizing tuberin cell expression. **Conclusions:** Retention of both hamartin and tuberin expression in brain lesions of Tuberous Sclerosis Complex, suggests allelic haplo-insufficiency or disruption of phosphorylative inhibition over the TSC1/TSC2 complex, caused by changes in various modulating proteins involved in cell growth and cell division control, as sufficient conditions to cause hamartomatous development in CNS structures.

NF1 pathogenesis: Four types of neurofibromas defined by components of the nerve sheath. *V. Riccardi.* The Neurofibromatosis Inst., La Crescenta, CA.

While clinical and natural history delineations of 4 types of neurofibromas (nfs) of NF1 are widely accepted, a pathogenetic substantiation of them has been lacking. Based on histopathology and ultrastructural studies of developing and mature peripheral nerves, in vitro reconstruction experiments and strategic utilization of knockout mice models, the importance of the nerve sheath in determining the nature of each nf can now be specified, providing a cogent underpinning of the 4 types of NF1 nfs, which in turn will be critical for further natural history and treatment protocols. The schema respects that there are 4 major elements to the nerve sheath, namely, the epineurium (EP), perineurium (PN), endoneurium (EN) and the Schwann cell (SC). Cutaneous nfs are **Endoneurial nfs**: no elements of the PN or EP contribute to the nf. Subcutaneous nfs are **Perineurial Simplex nfs**: single fascicles of a nerve give rise to a nf encapsulated by a well-defined PN. Nodular Plexiform nfs are **Perineurial Multiplex nfs**: multiple nerve fascicles in close proximity within a nerve form dense clusterings of PN-encapsulated nfs. Diffuse Plexiform nfs are **Epineurial nfs**: the EN breaches the PN and is only partially contained by the EP, the ultimate lack of EP containment allowing for encroachment on and replacement of surrounding normal tissue. The SC contributes to all four nfs, although endoneurial (cutaneous) nf SCs generally do not manifest loss of heterozygosity for *Nf1*. Thus, NF1 nfs are not accounted for simply in terms of a tumor suppressor gene model. Rather, a histogenesis control gene model is most likely to be informative about pathogenesis and potential treatments.

Interphase FISH screening for the LCR-mediated common rearrangement of isochromosome 17q in primary myelofibrosis. *G.A. Bien-Willner¹, P. Stankiewicz¹, J.R. Lupski^{1,2,3}, J.K. Northup⁴, G.V.N. Velagaleti⁵.* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Department of Pediatrics, Baylor College of Medicine, Houston, Texas; 3) Texas Childrens Hospital, Houston, Texas; 4) Department of Pathology University of Texas Medical Branch, Galveston, Texas; 5) Department of Pediatrics, University of Texas Medical Branch, Galveston, Texas.

Non-allelic homologous recombination (NAHR) between low copy repeats (LCRs) has been implicated recently in somatic rearrangements including isochromosome i(17q), which is associated with hematologic malignancies as well as solid tumors. In hematological malignancies, the most common i(17q) breakpoint results from LCR-mediated NAHR that creates a dicentric chromosome, idic(17)(p11.2). We report an elderly patient who presented with primary myelofibrosis (MF) with myeloid metaplasia (MMM), associated with idic(17)(p11.2) as the sole chromosomal abnormality. The rearrangement breakpoint maps to the previously defined LCR cluster, further suggesting that the genomic architecture of proximal 17p may be responsible for the formation of the majority of i(17q) cases. We developed a rapid screening test using interphase FISH to detect idic(17)(p11.2), discuss the potential prognostic value of this molecular diagnostic test, and examine the relevance of LCR-mediated NAHR to common rearrangements in neoplasms.

Characterization of a Population Polymorphism within cytoband 14q12 using high resolution BAC microarray CGH analysis. *I. Braude¹, B. Vukovic¹, S.K. Watson², W.L. Lam², J.A. Squire¹.* 1) Ontario Cancer Institute, Toronto, Ontario, Canada; 2) BC Cancer Agency, Vancouver, BC, Canada.

Array comparative genomic hybridization (aCGH), allows for a vastly improved resolution (as low as 80kb) compared to conventional CGH (5-10 MB), by using a series of contiguous BAC clones in a region of interest or across the entire human genome. Segmental duplications (duplicons), which account for ~5% of the genome, have been implicated in genomic alterations such as duplications, deletions, and inversions and are thought to be facilitating chromosomal translocations leading to cancer. Furthermore, duplicons that have been studied on detail have been shown to be highly polymorphic in copy number distributions in human populations. aCGH profiling of 4 CML cancer patients using a 1-2MB resolution BAC array identified a BAC clone within cytoband 14q12 that exhibited allelic gain on one homologue in 3 of 4 individuals. This genomic imbalance was confirmed using quantitative PCR (QPCR). To further characterize this observation, we analyzed a series of 14 normal individuals and 66 hematologic and pediatric neoplasms. Normal individuals, as well as 2 Chronic Myeloid Leukemia (CML) patients on whom both aCGH and QPCR were performed, were analyzed using quantitative FISH (QFISH). QFISH analysis identified the gain to be the result of a duplication of the signal on a single chromosome in both CML patients. Additionally, 3 of 14 normal individuals showed duplication of this 14q12 specific BAC. QPCR analysis of 18 of 64 neoplasms had allelic copy number imbalance. No statistically significant difference between the incidences of gain in normal individuals and cancer patients was seen. Locke et al. recently reported on the presence of duplicons in BAC clones used for aCGH. They suggest that duplicons may mimic events occurring elsewhere in the genome thereby yielding erroneous results. The results of our experiments suggest that duplicons may affect aCGH analysis and thus must be taken into account when designing the microarray as well as for accurate interpretation of aCGH results.

Chromosomal instability and cancer predisposition in Bub1 haploinsufficient mice. *D.V. Carrion¹, W. Zhang¹, P. McDonald¹, M. Lia², J. Heyer³, R. Kucherlapati¹*. 1) Harvard-Partners Center for Genetics and Genomics, Harvard Medical School, Boston, MA; 2) Institute for Cancer Genetics, Columbia University, New York, NY; 3) GenPath Pharmaceuticals, Cambridge, MA.

Genomic instability is the most common hallmark of human cancer. The loss of the mitotic checkpoint control has been thought to be responsible for genomic instability. Bub1 is a gene involved in the mitotic checkpoint control and mutated in some cancers. To study the role of this gene in cancer and genome instability we generated mice that carry a null mutation in Bub1. Homozygous mutant mouse embryos were detected at E3.5 but not at E8.5. We examined chromosome segregation in Bub1^{+/-} and wild type (WT) ES cell lines. At different passages the Bub1^{+/-} line showed a higher percentage of aneuploid cells. To confirm this observation FISH analysis was performed in blood lymphocytes of WT and Bub1^{+/-} mice using probes for chromosomes 9 and 17. Bub1^{+/-} mice showed a higher percentage of aneuploid cells ($P < 0.0001$). Bub1 heterozygous mice are fertile and show a tumor susceptibility phenotype late in their lives. Analysis of Bub1^{+/-} liver tumors by microarray-based comparative genome hybridization (aCGH) showed high chromosomal instability. These findings show that Bub1 haploinsufficiency leads to chromosomal instability in vivo and that this degree of instability predisposes the mice to develop tumors. Our results suggest that the Bub1 gene is essential for normal survival, chromosomal stability and tumor suppression.

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Novel Genetic Alterations in Early Stage Lung Cancer. *C. Garnis, J. Davies, E. Vucic, C. MacAulay, S. Lam, W. Lam.* Cancer Genetics, BC Cancer Research Center, Vancouver, British Columbia, Canada.

Lung cancer is the leading cause of cancer deaths in the United States. Despite advances in lung cancer treatment the overall 5 year survival rate for those diagnosed with lung cancer is bleak. In order to provide new clinical strategies that will ultimately result in increase survival rate we must determine the genetic alterations that drive this disease. The objective of this study is to identify novel genetic alterations in the progression of squamous cell carcinoma. Using a bacterial artificial (BAC) array comprised of 32,433 near over-lapping BAC clones that span the entire human genome we profiled microdissected CIS and invasive squamous cell lung cancer samples. Detailed comparison of a panel of pre-invasive and invasive samples revealed numerous recurrent sub-megabase alterations that are likely causally involved in lung neoplastic development. These include regions containing known oncogenes/tumor suppressor genes such as c-myc and cyclinD1 as well as novel sub-megabase regions containing only one or two genes. Coupling pre-invasive lesions with arrayCGH technology provides the most comprehensive and informative analysis from which candidate genes can be selected. This work was supported by funds from the Genome Canada/BC and the NCIC Terry Fox New Frontiers Program.

eIF-5A2 functions as an oncogene in the development of ovarian cancer. *X-Y. Guan¹, J.M. Fung¹, N-F. Ma¹, S.H. Lau¹, D. Xie¹, C.H. Tzang², M. Yang², J.S.T. Sham¹.* 1) Dept Clinical Oncology, Univ Hong Kong, Hong Kong, Hong Kong; 2) Department of Biology and Chemistry, The City University of Hong Kong, Hong Kong, China.

Ovarian cancer is the leading cause of death from female gynecological malignancies in developed countries and its incidence has been increasing in Asian Countries such as China and Singapore recently. Recurrent chromosomal changes in ovarian cancer have been well studied by comparative genomic hybridization (CGH) and amplification of 3q26 is one of the most frequent alterations. Recently, we have isolated a candidate oncogene eIF-5A2 from 3q26.2 using chromosome microdissection and hybrid selection. Sequencing analysis showed that eIF-5A2 shares a significant sequence homology (126/153, 82% amino acid identity) to eukaryotic initiation factor 5A (eIF-5A), including the domain needed for hypusine modification and the lysine-50 residue where the hypusine residue can be formed by posttranslational modification. The proliferation-related function of eIF-5A supports that eIF-5A2 is a candidate oncogene related to the development of ovarian cancer. In this work, the tumorigenic ability of eIF-5A2 was demonstrated by anchorage-independent growth in soft agar and tumor formation in nude mice. Furthermore, antisense DNA against eIF-5A2 could inhibit cell growth in ovarian cancer cell line UACC-1598 with amplification of eIF-5A2 in form of double minutes (DMs). Cell growth rate in UACC-1598 was also inhibited when the expression level of EIF-5A2 was decreased by the reduction of the copy-number of DMs. cDNA microarray study revealed that the oncogenic role of eIF-5A2 could be through WNT signal pathway. The correlation of EIF-5A2 overexpression and clinical features of ovarian cancer was investigated using tissue microarray and the result showed that eIF-5A2 overexpression was significantly associated with the advanced stage of ovarian cancer. These findings suggest that eIF-5A2 plays important roles in ovarian pathogenesis.

Amplification of the Steroidogenic Factor 1 (SF-1) Gene in Childhood Adrenocortical Tumors. *B. Haddad*¹, *L. Cavalli*¹, *M. Pianovski*², *I. Lalli*³, *R. Sandrini*², *R. Ribeiro*^{4, 5}, *G. Zambetti*⁴, *L. DeLacerda*², *G. Rodrigues*², *B. de Figueiredo*². 1) Georgetown Univ., Washington, DC; 2) Federal Univ. of Parana, Curitiba PR, Brazil; 3) Univ. Louis Pasteur, Illkirch, France; 4) St. Jude Children's Res. Hospital, Memphis TN; 5) Univ. of Tennessee, College of Medicine, Memphis, TN.

The incidence of childhood adrenocortical tumors (ACT) is 10-15 times greater in Southern Brazil than worldwide. The reasons for this increase and the genetic basis of this disease remain elusive. To further characterize the genetic changes in childhood ACT, we recently showed that gain/amplification of the 9q34 chromosomal region detected by CGH, is common in these tumors. Other subsequent studies involving both childhood and adult ACT have corroborated our findings. To follow up on these results, we have examined whether the Steroidogenic factor 1 (SF-1) gene located in this chromosomal region, is amplified in childhood ACT. SF1 is an orphan member of the nuclear receptor family of transcription factors. It plays an important role in the development and function of the adrenal cortex. We designed a FISH probe consisting of a BAC clone containing sequences of the SF-1 gene and used FISH to detect SF-1 copy number changes in a cohort of nine pediatric ACT cases where we had previously detected a consistent gain of 9q34 in 8 of them using CGH. Our FISH analysis was in full agreement with our CGH findings. Specifically, all 8 patients who showed copy number gain of 9q34 by CGH, showed increased copy number of the SF-1 gene by FISH. This is the first study showing an association between an increased copy number of SF-1 and ACT. The significance of this observation needs to be further investigated in both pediatric and adult ACT. Because this gene is detected at the earliest stages of development of the urogenital ridge, where both the gonads and the adrenal cortex are derived, and because this expression is maintained throughout development up till adult life, further studies examining the mechanisms and consequences of the amplification of this gene in ACT are critical to a better understanding of the onset and progression of this disease.

The somatic QMPSF : an efficient tool to detect genomic rearrangements in cancer. *A. Killian¹, F. Blanchard², A. Lamy², F. Le Pessot², J. Mtayer², T. Frebourg¹, J-M. Flaman¹, R. Sesboüé¹.* 1) Inserm U614, Faculty of Medicine, 76183 Rouen, France; 2) Department of Pathology, Rouen University Hospital, 76031 Rouen.

The majority of the malignant tumors exhibit a strong chromosome instability (CIN) that contributes to the malignant transformation, through the loss of tumor suppressor genes and activation of oncogenes. It is therefore essential to identify genes whose alterations are responsible for this genetic instability. Using an original biological screen in yeast based on a CIN indicator strain, we identified candidate genes whose inactivation or activation disturbs the correct segregation of the chromosomes. These genes encode mainly cell cycle regulators, phosphatases and proteins involved in ribosome biogenesis. In order to look for genomic rearrangements (loss of heterozygosity (LOH), or gain of gene copy numbers) of these candidate genes in primary aneuploid tumors, we developed a simple molecular assay based on the QMPSF (Quantitative Multiplex PCR of Short fluorescent Fragments), a method initially designed to detect germline genomic rearrangements. The QMPSF is based on the simultaneous amplification of multiple target sequences, using dye labeled primers in conditions that allow rapid and reliable quantitative comparison of the fluorescence of each amplicon. For each patient, the somatic QMPSF profile generated from the tumor is superimposed to that generated from the non tumoral tissue. The somatic QMPSF allows the simultaneous analysis of 10 different genes. The somatic QMPSF, performed on 30 primary tumours, revealed recurrent somatic rearrangements of genes involved in ribosome biogenesis. The somatic QMPSF is a simple, cheap and sensitive method to detect loss or gain of genetic material in cancer. Furthermore, its flexibility should facilitate the mapping of recurrent somatic genomic rearrangements observed in malignant tumours.

The effects of low dose gamma radiation in Trp53 heterozygous cancer prone mice: Genomic instability and Loss of Heterozygosity. *J. Lavoie¹, J-A. Dolling², R.E. Mitchel³, R. Carter⁴, D.R. Boreham¹.* 1) Medical Physics, McMaster University, Hamilton, ON, Canada; 2) The Credit Valley Hospital, Mississauga, ON, Canada; 3) Atomic Energy of Canada Limited, Chalk River, ON, Canada; 4) Health Sciences Center, McMaster University, Hamilton, ON, Canada.

The Trp53 gene is clearly associated with increased cancer risk. This, coupled with the broad understanding of its mode of action at the molecular level, makes this gene a good candidate to study the relationship between genetic risk factors and spontaneous cancer occurring in mice exposed to low dose radiation. We have shown that adaptive responses to low dose could increase cancer latency, as well as lifespan. These end points were used to assess changes in the risk in Trp53 heterozygous mice. To understand the molecular processes that influence cellular risk, spectral karyotyping (SKY) was used to detect chromosomal stability, and the loss of heterozygosity (LOH) at the Trp53 locus was evaluated using Rolling Circle Amplification (RCA). Mice were gamma-irradiated with daily doses of 0.33 mGy for 73, 143, 217, 295, 331 or 366 days, corresponding to total body doses of approximately 2, 5, 7, 10, 11 or 12 cGy. For each mouse exposed to 11 and 12 cGy and their unexposed littermates, 100 metaphases were analyzed using SKY and virtually no structural anomalies were observed in any group. This indicates that the presence of a defective copy of Trp53 does not appear to affect either spontaneous or chronic low dose induced chromosomal instability in cancer prone mice. To evaluate LOH, detection of the NEO and the Trp53 alleles using the dual color RCA was utilized. In our hands, this protocol did not give the required sensitivity. Therefore, a quantitative real-time PCR experiment using the LightCycler instrument was designed to target both alleles. More than 800 QRT-PCR reactions were conducted on DNA extracted from tissues of the irradiated and unexposed mice. A statistical analysis of the data is in progress to confirm any changes in the original number of DNA copies. This research will provide information regarding the health effects and cancer risk of low doses of radiation in genetically susceptible individuals.

Immunohistochemical analysis of P53, CYCLIN D1, RB, C-FOS and N-RAS genes _expression in hepatocellular carcinoma in Iran. *E. NOBAKHT HAGHIGHI¹, M.R. Zali¹, S.J. Mirhassani Moghaddam², S. Dadgar⁴, S. Samiee¹, N. Shahid¹, A.R. Keramati¹, H. Najmabadi³.* 1) RCGLD, Taleghani Hospital, Evin Ave, TEHRAN, Iran; 2) The University of Texas MD Anderson Cancer Center Pulmonary Research Lab, 2121 Holcombe Blvd, Houston, TX Email: smoghadd@mdanderson.org; 3) The Socail Welfare and Rehabilitation Sciences University; 4) Banting And Best Department of Medical Research, University of Toronto, Canada.

Purpose: The effects of some genes have been shown in development of hepatocellular carcinoma in several studies but there are some controversies about them yet. This study was undertaken to analyze simultaneous status of p53, cyclin D1, Rb, C-fos and N-ras genes in Iranian patients. **Methods:** The paraffin-embedded tissue samples of 25 patients (18 male and 7 female) with documented hepatocellular carcinoma were collected from 22 pathology centers in Tehran one year (2001). Using immunohistochemistry method (Avidin-Biotin-Peroxidase), they were stained for detection of p53, cyclin D1, Rb, C-fos and N-ras proteins accumulation. **Results:** All of 25 tumors were in grade I (well differentiated). Six (24%), 5(20%), 13(52%) and 2(8%) were positive for p53, cyclin D1, C-fos and N-ras, respectively. Besides, 22(88%) cases showed complete loss of Rb protein _expression. Comparing p53 positive cases with the negative ones, the former group showed a higher (9 times) risk of being positive for Rb gene. This figure was 2.66, 2.75 and 3.6 for cyclin D1, C-fos and N-ras, respectively. Rb loss of _expression in association with p53 over-_expression has been observed in 4(16%) of samples. This figure was 5(20%) for Rb and cyclin D1, 2(8%) for Rb and N-ras and 11(44%) for Rb and C-fos. Comparing cyclin D1 positive cases with the negative ones, the former group showed a higher (2.85 and 4.75 times) risk of being positive for C-fos and N-ras genes. **Conclusion:** Like previous studies, the development of mutation in some of these genes especially C-fos, Rb and p53 appears to have a key role in the carcinopathogenesis of hepatocellular carcinoma in Iran. Also a significant association between the simultaneous mutations of some of these genes during development of this cancer is likely.

The Role of *FANCD2* in the DNA Damage Response of Oral Cancer Cell Lines. *E.M. O'Leary*¹, *K. Mitchell*², *R.A. Parikh*², *J.S. White*^{2,3}, *S.M. Gollin*^{2,3}. 1) Genetic Counseling Program, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA; 2) Human Genetics, University of Pittsburgh, Pittsburgh, PA; 3) University of Pittsburgh Cancer Institute, Pittsburgh, PA.

The genetic mechanisms that contribute to Head and Neck Squamous Cell Carcinoma (HNSCC) and Oral and Oropharyngeal Squamous Cell Carcinomas (OSCC) are incompletely understood. Cancer predisposition is associated with chromosome instability and hypersensitivity to DNA damaging agents. Fanconis Anemia patients are extremely sensitive to cross-linking agents and are at increased risk to develop several cancers including leukemias, gynecological and oral cancers. The Fanconis Anemia proteins (FANCs) are thought to be involved in pathways necessary for repairing cross-linked DNA. Six FANC proteins (A, B, C, E, F and G) assemble in a nuclear core complex and two other identified proteins, *FANCD1/BRCA1* and *FANCD2* act downstream of the complex. This core complex is required for the monoubiquitination of the *FANCD2* protein to the *FANCD2-L* isoform in response to DNA damage. To determine if mutations in FANC genes may contribute to head and neck cancer, cell lines were treated with the clastogenic cross-linking agent, DEB, to quantify the number of double strand breaks induced. Based on the association of Fanconis Anemia and OSCC, we expected to see similarities among the treated oral cancer cell lines and the cell lines from FA patients when treated with DEB. *FANCA*^{-/-} (GM06914) and *FANCD2*^{-/-} (GM16633) cell lines were analyzed as positive controls and normal fibroblasts were used as negative controls in demonstrating the inability of HNSCC cells to repair DNA damage. The tumor cell lines treated with DEB have a increased number of double strand breaks, leading to the formation of tri- and quadrads, which is indicative of defects in the DNA damage response. Immunoblots with a *FANCD2* antibody show differential activation of *FANCD2-L* in the presence of this particular DNA cross-linking agent.

***ATR* gene abnormalities in squamous cell carcinoma of the head and neck.** R. Parikh¹, J. White^{1,4}, D. Schoppy^{1,3}, R. Bhaskaran², S. Gollin^{1,4}. 1) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Molecular Genetics and Biochemistry, University of Pittsburgh, Pittsburgh, PA; 3) Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA; 4) University of Pittsburgh Cancer Institute, Pittsburgh, PA.

Squamous cell carcinoma of the head and neck (SCCHN) at oral and oropharyngeal sites is the sixth most common cancer worldwide. A wide variety of genetic and epigenetic defects are seen in the tumor cells. Amplification of *CCND1* gene located in 11q13 as a homogenously staining region (hsr) and a gain of distal 3q are seen in nearly 45% of SCCHN. The *ATR* gene maps to chromosomal band 3q22-24 which is frequently gained in different types of cancers. *ATR* is a serine threonine kinase and a critical regulator of cellular responses to DNA damage. It phosphorylates and activates different substrates like c-Abl, p53, Nbs1, Smc1 and Chek1 in response to UV radiation and cross-linking agents. *ATR* also plays an important role in maintenance of integrity of fragile sites. We performed dual color FISH using probes for *ATR* and chromosome 3 centromere (CEP3) on eight SCCHN cell lines. We found copy number gain or structural rearrangement of *ATR* gene in five out of the eight SCCHN cell lines. Three cell lines showed a gain (*ATR*/CEP3 ratio >2.0) associated with isochromosome 3q formation. This correlates with over expression of the *ATR* protein and loss of p53 expression in these cell lines detected by immunoblotting. We hypothesize that *ATR* overexpression (caused by an increase in copy number or chromosomal rearrangements) is an important event which may contribute to the development and progression of carcinoma. Our studies also show loss that of p53 expression is associated with overexpression of *ATR* though the exact relationship between the two needs to be elucidated.

ABERRATIONS AT 8P12 IN BREAST CANCER. *A.A. Shadoo^{1,2}, R.J. deLeeuw^{1,2}, V.S. Lestou^{2,3}, D.G. Huntsman^{2,3}, W.L. Lam^{1,2}.* 1) Cancer Genetics, British Columbia Cancer Research Centre, Vancouver; 2) Pathology and Laboratory Medicine, University of British Columbia, Vancouver; 3) Genetic Pathology Evaluation Centre, Vancouver Hospital and Health Sciences Centre and British Columbia Cancer Agency.

Background: Breast carcinoma is the most frequently diagnosed cancer and is one of the leading causes of cancer related deaths in women. Recurrent chromosomal translocations involving the *NRG1* gene located at 8p12 have been observed in multiple breast cancer cell lines. However, the relevance of this translocation in clinical breast cancers remains unclear. Fluorescence in situ hybridization (FISH) has identified amplified *NRG1* in a number of invasive clinical breast cancer specimens, raising the possibility that *NRG1* disruption is more complex than a simple translocation event. **Hypothesis:** 1) Alterations at 8p12 will be present in clinical specimens. 2) The increase in *NRG1* copy number observed in breast cancer specimens is related to a translocation event with the breakpoints at 8p12 potentially disrupting the gene. **Experimental Design and Results:** Five archival specimens with amplification of the *NRG1* locus identified through FISH using Tissue MicroArray (TMA) were selected for this study. Isolated sample and pooled female genomic reference DNA were differentially labelled and hybridized to SMRT arrays containing approximately 32,000 BACs. These arrays provide overlapping coverage of the human genome while using 600ng of specimen DNA. CGH imaging and analysis was done using a charge coupled device (CCD) based scanner and associated analysis software. Visualization was accomplished using custom software called SeeGH. FISH results were confirmed in all cases and boundaries of amplification were identified. **Conclusion:** Using the newly developed Sub-megabase Resolution Tiling Set (SMRT) array for comparative genomic hybridization (CGH), we have identified the specific boundaries of amplification at the *NRG1* gene region on chromosome 8p.

Analysis of prostate cell lines and microdissected tumors using tiling resolution whole genome analysis. *S.K. Watson¹, J.A. Squire², B. Beheshti², W.L. Lam¹*. 1) Cancer genetics, BC Cancer, Vancouver, BC, Canada; 2) Cellular and Molecular Biology, Ontario Cancer Institute, Toronto, Ontario, Canada.

The advent of a tiling resolution BAC CGH array has increased the resolution of DNA amplification or deletion analysis allowing copy number analysis of the entire genome in a single experiment. In this study we looked at well identified cell lines such as DU 145, PC3 and LnCaP as well as Gleason 3 or 4 grade laser capture micro-dissected tumor samples. This high resolution technique allows rapid identification of DNA irregularities that can be easily linked to prostate cancer profiling. Through array CGH analysis it is possible to detect single copy alterations within the genome when compared to a diploid reference sample. Using this technique, common prostate cancer cell lines showed unique patterns allowing potential trend recognition of prostate cancer progression. The array consisted of 32,433 BAC clones spotted in triplicate on two slides. As conventional CGH allows only 10-20MB resolution this array represents a 100-200 fold increase over traditional methods. Six cell lines commonly used to model prostate cancer were individually comparatively hybridized against normal male reference DNA to determine alterations in the genome. 200ng of sample and reference DNA were random primed with Cy3-dCTP or Cy5-dCTP fluorochromes and hybridized to the slides in the presence of COT-1 DNA. The whole genome array allowed identification of numerous novel and previously reported regions not described in these prostate cancer cell lines. Single copy deletions and amplifications were detectable and identifiable as confirmed by FISH and SKY karyotyping. Comparison of the sub-megabase tiling path resolution tiling (SMRT) whole genome array CGH profiles for commonly used prostate cell lines will allow rapid recognition of putative genes involved in prostate pathogenesis. This combined with the clinical sample data may offer a better understanding of prostate cancer progression globally within the genome. This work was supported by funds from Genome Canada/Genome BC.

Phosphorylation of Histone Variant H2AX as a Marker for DNA Damage Pathway Competency in Oral Cancer Cells. *J.S. White*^{1,2}, *R.A. Parikh*², *D.S. Schoppy*^{2,3}, *S.M. Gollin*^{1,2}. 1) University of Pittsburgh Cancer Institute, Pittsburgh, PA; 2) Department of Human Genetics, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA; 3) Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA.

A subset of head and neck squamous cell carcinomas (HNSCC) and Oral and Oropharyngeal Squamous Cell Carcinomas (OSCC) are characterized by amplification of chromosomal band 11q13, which is thought to be driven by cyclin D1 (*CCND1*) overexpression. As a result of this process, the genes distal to 11q13 are often lost, a number of which are involved in the DNA damage response. One of these genes, *H2AFX*, is a histone variant thought to be critically important to the recognition of DNA strand breaks. Other genes distal to the amplicon include *ATM*, *CHEK1*, and *MRE11A*, all of which are involved in the damage repair response. We hypothesize that haploinsufficiency, or partial insufficiency, of the DNA repair genes distal to 11q13 would lead to increased susceptibility of the cell lines to DNA damage. Fluorescence *in situ* hybridization (FISH) was used to confirm the presence of the 11q13 amplicon, and the copy number of the chromosome 11 centromere (CEP11) and the *H2AFX* gene at 11q23.2-23.3. FISH results indicate that four cell lines have amplification of 11q13, and loss of genes distal to that region. Five cell lines show distal loss on the long arm of chromosome 11, and the remaining two cell lines have no alterations on chromosome 11. Using phosphorylation of H2AX as a surrogate marker of a proficient DNA damage response, we evaluated the competency of DNA repair in eleven OSCC cell lines after treatment with (10Gy) ionizing radiation (IR) or (20 J/m²) ultraviolet (UV) exposure by formation of -H2AX foci. Preliminary foci data indicate an incomplete response to IR and UV. Immunoblots of the damage response genes showed differential expression between cell lines. Inadequate response to either IR or UV is expected to be critical to the phenotype of these cells. Using these cell lines as models for the original tumor, these studies may indicate patient response to treatment, environmental or other exogenous exposures.

Breast and ovarian cancer screening practices and prophylactic surgery in at risk women: an Australian multicenter study. *Y.C. Antill¹, T. Dudding², T. Hall³, J. Kirk⁴, J. Reynolds¹, K. Tucker⁵, S. Wong⁶, MA. Young¹, KA. Phillips¹.* 1) Peter MacCallum Cancer Centre, VIC; 2) Hunter Genetics, NSW; 3) Monash Medical Centre, VIC; 4) Westmead Hospital, NSW; 5) Prince of Wales Hospital, NSW; 6) Royal Melbourne Hospital, VIC, Australia.

This study examined screening for breast and ovarian cancer, and prophylactic surgery (PS) undertaken by women at moderate or high risk for hereditary breast and ovarian cancer following risk management consultation at a Family Cancer Center (FCC). A self-report questionnaire was mailed to 396 women with no personal history of cancer. They had attended one of six FCCs, a median of 3.63 years prior. Questions included; screening or PS undertaken, risk perception and cancer specific anxiety. Family history, level of risk and screening recommendations given were abstracted from subjects medical record. Fishers exact test or the exact form of the Mantel-Haensel test was used to investigate associations between adherence and each factor. Ordinal logistic regression was used to investigate associations between continuous-scale covariates and adherence. 182/266 (68.4%) responded. 130 were lost to follow-up. Screening adherence was: breast self exam 39%, clinical breast exam 44%, mammogram 65%, transvaginal ultrasound 37%, CA125 64%, (no difference between risk groups). Predictors of adherence included; older age, BRCA1/2 positive, having an affected first degree relative (FDR), higher risk perception, and greater income (all $p < 0.05$), but these associations were not consistent across all screening strategies. Predictors of over-screening were; higher cancer specific anxiety and/or a mutation. Low levels of cancer anxiety predicted for under-screening. 16/182 (9%) respondents had undergone PS: mastectomy (7), oophorectomy(10). Predictors of PS were; at least one affected FDR, or cancer death of a FDR. Despite specific advice, women at risk for breast and ovarian cancer were mostly nonadherent to the recommendations for screening with low and high levels of anxiety having a negative influence on compliance. Further studies are needed to evaluate interventions that might improve screening adherence.

Defective splicing is not a major consequence of missense mutation in HNPCC. *J. Auclair¹, M-P. Buisine², C. Lasset³, F. Desseigne⁴, V. Bonadona³, J-C. Saurin⁵, A. Puisieux¹, Q. Wang¹.* 1) Unit d'Oncologie Molculaire, Unit INSERM 590, Centre Lon Brard, Lyon, France; 2) Laboratoire de biochimie et biologie molculaire, CHU Lille, France; 3) Unit de prvention et d'pidemiologie gntique, Centre Lon Brard, Lyon, France; 4) Dpartement de mdecine, Centre Lon Brard, Lyon, France; 5) Service de Hpathogastroenterologie, Centre Hospitalier Lyon Sud, France.

HNPCC is a common cancer syndrome with the predisposition to colorectal cancer and some extracolonic cancers and is caused by germline mutation of one of DNA mismatch repair genes. Up to 30% of the mutations identified so far affect only one nucleotide, making the pathogenic effect determination difficult. Recent studies have shown that nucleotide substitutions can alter pre-mRNA splicing when they affect elements essential to exon recognition called ESE (Exonic Splicing Enhancers). Recently, Gorlov et al (2003) reported that an excess of presumed deleterious missense mutations of hMLH1 and hMSH2 genes in HNPCC families were located in predicted ESE sites (ESEfinder), suggesting that the disruption of ESE sites was the cause of their deleterious effect. For evaluating the role of ESE, we screened 20 hMLH1 and 16 hMSH2 variants for aberrant splicing. 60% were located at sequences predicted as the target of at least one of the SR proteins with a threshold value higher than 2.5 (ESEfinder). Five additional variants affecting consensus exon-intron splicing sites were used as controls. RT-PCR were performed using the RNA isolated from lymphoblastoid cell lines after puromycin treatment. Our results revealed that all variants affecting exon-intron consensus sites generated aberrant splicing, whereas none but one ESE-unrelated variant gave rise to abnormal splicing. Our results suggest that although a number of sequences are recognizable by one of the SR proteins, their role in splicing control seems to be limited when individually affected. Location at such predicted ESE sites should not be considered determinant for interpreting pathogenic effect of missense mutations in genetic consultation, before confirmation at the RNA level.

A 25 year perspective of the DEB test for Fanconi anemia: correlation of mosaicism with clinical outcome. A.D. Auerbach¹, Y. Flit¹, S.D. Batish¹, J. Morales¹, M. Berwick². 1) Lab. Human Genetics & Hematology, The Rockefeller Univ., New York, NY; 2) Dept. Medical Epidemiology, Univ. of New Mexico, Albuquerque, NM.

Fanconi anemia (FA) patients are characterized by a cellular hypersensitivity to DNA cross-linking agents and cancer predisposition. Diepoxybutane (DEB) test results were analyzed for validation with 523 subjects diagnosed as affected and 2097 diagnosed as unaffected. Subjects were tested if they had bone marrow failure (BMF), congenital malformations (CM), BMF + CM or were siblings of FA patients. Peripheral blood lymphocytes were cultured with 0.1g/ml DEB according to a protocol previously described (Auerbach et al., Blood 73:391-396, 1989). Affected and unaffected groups had 8.5 (SD=4.6) and 0.01 mean breaks/cell (SD=0.03) respectively. 98.9% of patients diagnosed as FA and none of the patients diagnosed as non-FA had greater than 0.65 mean breaks/cell. 96.6% of non-FA subjects and 0% of FA patients had less than 0.09 mean breaks/cell. Thus only a small number of affected individuals could not be discriminated on the basis of mean breaks/cell. These mosaic patients have two populations of lymphocytes: (i) aberrant cells with breaks and exchanges typical of DEB-treated FA cells and (ii) non-aberrant cells with no breaks. For FA and non-FA subjects, the mean % aberrant cells was 92 and <0.01 respectively. Most FA patients (98%) had >18% aberrant cells; 2% had between 1-18%. Among non-FA subjects, 74% had 0 aberrant cells, while 26% had between 1-18% aberrant cells. About 10% of FA patients have 50% or less aberrant cells. We next analyzed data for 673 non-mosaics (>50% aberrant cells) and 77 mosaics for clinical outcome. There are 246 deaths among non-mosaics and 22 deaths among mosaics. Using Kaplan-Meier estimates, the median age at death for non-mosaics is 22.8 years and the median age at death for mosaics is 32.1 years (log-rank $p < 0.01$). There are 133 non-mosaics with malignancy (including MDS) and 18 mosaics. By Kaplan-Meier estimates, the median age at cancer for non-mosaics is 33.4 years and for mosaics 37.7 years ($p = 0.37$). Thus, mosaics live significantly longer than non-mosaics but there is no evidence of a difference in cancer incidence between the two groups.

Direct mutation detection in breast and ovarian cancer susceptibility genes using Meta/PCR. *B. Azimifar*^{1,2}, *M. Eram*^{1,2}, *V. Lotfi*², *M. Masrouri*², *P. Folady*², *S. Zeinali*^{1,2}. 1) Biotechnology Research Center, Biotechnology Research Center, Pasteur Institute of Iran, Pasteur St., Tehran, Iran; 2) Medical Genetic Lab of Dr Zeinali.Fatemi Square, bisetoon St, Leon Building, Tehran, Iran.

Approximately 5-10% of breast cancer and ovarian cancer cases are thought to be associated with an autosomal dominant pattern of inheritance, usually relates to mutations in the BRCA1 and BRCA2 genes. The lifetime risk of eventually developing breast cancer accumulates to 55-90% and 44-80% by age 70 for carriers of BRCA1 and BRCA2 mutations, respectively. Moreover, it is know thought that BRCA2 may account for as much as 10-35% of familial ovarian cancers. The BRCA1/2 genes are too large (each one is more than 80 KB in length) with 24 and 27 exons respectively. Many of these exons are too small and sequencing of them separately are time consuming and expensive. The majority of BRCA1/2 mutations also are unique and each family with a defined history of breast or ovarian cancer tends to have its own mutation. We used a new approach for mutation detection of BRCA1/2 genes which using Meta/PCR followed by direct sequencing. In this technique, we amplify each exon of BRCA1/2 separately with primers that each one has a tail in its 5' end. So that these amplicons could be used in a second PCR in which they link to each other to make longer fragments and so on. Then chimeric DNAs (instead of small exons of BRCA1/2 genes) could easily be used for sequencing which lead to rapid and accurate screening for mutation of BRCA1/2 genes. The results can be used as a important prognosis factor for following up the at risk members of family with inherited breast and ovarian cancers.

Development of a conditional knockout mouse model of the Birt-Hogg-Dubé syndrome. *M. Baba*¹, *L. Tessarollo*², *M.E. Palko*², *E. Southon*³, *M. Furihata*¹, *B. Zbar*¹, *L.S. Schmidt*³. 1) Laboratory of Immunobiology, NCI-Frederick, Frederick, MD, 21702; 2) Mouse Cancer Genetics Program, NCI-Frederick, Frederick, MD, 21702; 3) BRP, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD, 21702.

The Birt-Hogg-Dubé syndrome (BHD) is a rare inherited genodermatosis characterized by hamartomas of the hair follicle (fibrofolliculomas), spontaneous pneumothorax, and an increased risk of renal cancer. The BHD gene encodes a novel 579 amino acid protein, folliculin, with unknown function. Most mutations found in the germline of BHD patients cause frameshifts or premature termination codons predicted to truncate the BHD protein. Mutations in BHD gene orthologs were also found in naturally occurring hereditary multifocal renal cystadenocarcinoma and nodular dermatofibrosis in the German Shepherd dog and in the spontaneous Nihon rat model of inherited renal cancer. Homozygous mutations in the dog or rat model of BHD were embryonic lethal. To study BHD gene function in vivo we are developing a BHD mouse model. We have designed a conditional BHD gene targeting vector to avoid embryonic lethality and have constructed it by recombinogenic engineering. After electroporation with the BHD targeting vector, mouse ES cells were screened by Southern blotting and correctly targeted ES cell clones were injected into blastocysts. We will describe our method and report progress in establishing a conditional BHD knockout mouse model. Funded in part by DHHS#NO1-CO-12400.

Approaches to the identification of endometrial cancer patients at risk for HNPCC. *S.A. Babb, D.G. Mutch, M.A. Powell, R.K. Gibb, J.S. Rader, T.J. Herzog, A.J. Whelan, P.J. Goodfellow.* Washington University, St Louis, MO.

Early onset cancer (<50 years), second malignancies, cancer family history and tumor MSI are hallmarks of HNPCC. At present it is unclear what the best approach is to the identification of endometrial cancer patients with HNPCC. To address this we evaluated clinical and molecular features in large endometrial cancer cohort. 412 endometrial cancers from a single oncology clinic between 1994 and 2002 were assessed for MSI and MLH1 methylation. Detailed family histories were obtained for 164 patients (40%). Clinical and molecular features were similar for the 164 probands with detailed family history (FH) and the remaining 248 individuals (NoFH) with the exception of age of diagnosis. The FH group was younger than the NoFH group (62 versus 66 years, $p=0.0004$; student's t-test). However, the percentage of probands diagnosed before age 50 was not statistically different. Nearly 60% of the FH group reported a first or second-degree relative with an HNPCC-associated malignancy. The mean age of onset for those women was not different from those with no relatives with an HNPCC cancer. Twelve (7%) of the FH group and 5% of the NoFH group had a second HNPCC-associated cancer. The mean age at endometrial cancer diagnosis for those women with a second HNPCC malignancy was not statistically different than for women without a double primary. Among the 12 patients with a second HNPCC cancer, 9 (75%) had first or second degree relative with an HNPCC-associated tumor. Of the 152 women without a second HNPCC cancer, 87 (57%) had relatives with HNPCC tumors. Of the 12 HNPCC double primary cases, 4 were MSI positive and lacked MLH1 methylation and all had relatives with HNPCC-associated cancers. 5 of the 8 other double primary cases had relatives with HNPCC cancers. Only one of the double primary cases was diagnosed before age 50. Together, these data suggest that a combination of age of onset of endometrial cancer, synchronous or metachronous HNPCC-associated tumor, family history of HNPCC-associated tumors, and MSI and MLH1 methylation status may be the best approach to identifying endometrial cancer cases at increased risk for HNPCC.

Unique germline E-cadherin mutations in two Hereditary Diffuse Gastric Cancer families. *C. Baeslach¹, M. Harlan¹, K. Campbell¹, M. Ramirez¹, J. Palascak¹, C. Aronchick², P. Guilford³, B. Humar³, B.M. Boman¹.* 1) Thomas Jefferson University, Philadelphia PA; 2) Pennsylvania Hospital, Philadelphia PA; 3) University of Otago, New Zealand.

Introduction: 10% of gastric cancer has an autosomal dominant pattern that increases cancer risk. Criteria for Hereditary Diffuse Gastric Cancer (HDGC) are: 2 or more documented cases of diffuse gastric cancer in 1st or 2nd degree relatives, with 1 case before 50, OR 3 or more cases in 1st or 2nd degree relatives, independent of age. Germline mutations in CDH1, encoding for E-cadherin, have been found in 11-50% of HDGC families meeting clinical criteria. Presented are 2 HDGC families with unique CDH1 mutations. **Methods:** Family 1-The proband had genetic counseling due to a personal and family history of diffuse gastric cancer. She was diagnosed at 42 with diffuse gastric cancer, her father at 68, paternal aunt at 39, and paternal grandfather at 57. A 2nd paternal aunt had colon cancer at 55 and gastric cancer at 76 and a 3rd paternal aunt had breast cancer at 50. Family 2-The proband was diagnosed with gastric cancer at 36, sister at 30 and father at 33. His paternal grandfather had unconfirmed gastric cancer in his 70s. In both families, clinical criteria were met. **Results:** Family 1, genetic analysis revealed a germline mutation 49-2 AC at the splice receptor site for exon 2. This mutation is predicted to truncate the protein after codon 16. Family 2, genetic analysis of CDH1 revealed a germline mutation in exon 8 1137+1 GA. Although not functionally studied, this mutation is thought to affect splicing of E-cadherin. **Conclusion:** These families exhibit striking histories of diffuse gastric cancer. It is important to identify these families, offer testing, and find mutations. Unfortunately, only 25% of families who meet criteria have an identifiable CDH1 mutation. There are research labs (eg Univ of Otago) outside the US that offer testing to identify CDH1 mutations. The only certified clinical lab in the US must first have research testing and functional gene studies to proceed. As more families are identified with HDGC, genetic testing should lead to reduced mortality from gastric cancer.

Sequence analysis of Succinate Dehydrogenase Subunit A Gene (*SDHA*) for paraganglioma tumor susceptibility.

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Background: Germline heterozygous mutations in three of the four subunits (*SDHB*, *SDHC*, *SDHD*) of succinate dehydrogenase (SDH; mitochondrial complex II) cause majority of hereditary paragangliomas (PGLs). Thus far, no PGL mutations were described in the *SDHA* gene, which encodes the major catalytic subunit of the enzyme. It has also been suggested that there might be two *SDHA* isoforms encoded by distinct loci in the human genome. Therefore, we sought to determine role of *SDHA* mutations in paraganglioma susceptibility in the light of evidence that there might be multiple *SDHA* loci encoding functional protein products.

Methods: We have searched for mutations in the known *SDHA* locus on chromosome 5p15 by genomic sequencing of its 15 exons in subjects with familial/syndromic paragangliomas who did not reveal mutations in the other SDH subunits. The subjects included two cases with familial head and neck paragangliomas; two nonfamilial cases with Carney's triad, who had paragangliomas and gastric leiomyomas; and one nonfamilial case with bilateral paragangliomas, mild mental retardation and premature aging.

Results: No pathogenic mutations have been detected in exons 2-15. However, we have found nine expressed single nucleotide polymorphisms (SNPs) in a set of 15 unrelated chromosomes. Whereas six SNPs were synonymous, three SNPs predicted amino acid alterations. Two amino-acid-altering SNPs jointly predict an allelic variant that is identical to the proposed *SDHA* isoform, that was suggested to be encoded by a second intronless gene.

Conclusions: These findings suggest that a single highly polymorphic locus is responsible for sequence variations in *SDHA* transcripts and that this locus does not appear to play an important role in paraganglioma susceptibility. The full spectrum of allelic variations and the extent of linkage disequilibrium across the *SDHA* gene locus are under further investigation.

FAP-causing mutation in the APC gene that was missed by PTT was detected by DHPLC. S.D. Bercovich¹, T. Naiman¹, R. Shomrat¹, S. Simchoni¹, Y. Yaron^{1,2}, A. Orr-Urtreger^{1,2}. 1) Genetic Institute, Tel Aviv Sourasky Medical Ctr; 2) Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

Specific conditions for denaturing-high-performance-liquid-chromatography (DHPLC) analysis of the *Adenomatous Polyposis Coli (APC)* gene were developed and the sensitivity of this method was compared to direct genomic sequencing and protein truncation test (PTT). Genomic DNA was obtained from 36 Familial Adenomatous Polyposis (FAP) patients with known mutations, 35 patients suspected for FAP that were negative for mutation screening by sequencing and/or PTT (Gavert et al, *Hum Mutat.* 2002), 8 new cases and 50 normal controls.

The coding sequences and exon-intron boundaries of exons 1-14 and more than 3Kb of exon 15 were screened using a single DHPLC condition for all fragments. A novel 2031delCAGT frameshift mutation was identified in a patient diagnosed as FAP at the age of 18 years. The position of this mutation close to the origin of the PTT fragment generated a very short truncated protein that was missed by the PTT analysis. DHPLC also detected a missense mutation, A685G (I228V), that was not identified by bi-directional sequencing. Allele separation by sub-cloning and sequencing confirmed this mutation. Additional 4 nonsense and 4 missense mutations were identified in the *APC* gene. One of these missense mutations was localized 3 to a premature stop codon, suggesting that it is not the disease causing mutation, while the contribution of the other 3 missense mutations to FAP is yet unclear. We concluded that DHPLC of the *APC* gene is a reliable method for mutation screening in patients suspected for FAP.

MYH gene mutations in young onset patients (< 50 years old) with microsatellite stable (MSS) colorectal cancer(CRC). *L.A. Boardman¹, R.A. Johnson², L. Wang², K.J. Steenblock², G.M. Petersen³, A.J. French², L.J. Burgart², N.M. Lindor⁴, S.N. Thibodeau².* 1) Internal Medicine, Mayo Clinic College of Medicine, Rochester, MN; 2) Laboratory Medicine and Pathology; Mayo Clinic College of Medicine, Rochester, MN; 3) Health Sciences Research; Mayo Clinic College of Medicine, Rochester, MN; 4) Medical Genetics; Mayo Clinic College of Medicine, Rochester, MN.

Biallelic germline mutations in MYH, a base excision repair enzyme, recently were discovered to be causative for an autosomal recessively inherited syndrome of CRC and multiple adenomatous colon polyps. To date, the vast majority of biallelic germline mutations in MYH have been associated with the a phenotype of multiple adenomas ranging from 20 to 100s of polyps and felt to be an explanation for a sizeable portion of attenuated familial adenomatous polyposis not related to germline APC mutations. Because of the earlier age of onset of CRC associated with hereditary CRC syndromes we evaluated a young onset CRC population in which fewer than 3 lifetime adenomas were present. Here we report the results of germline MYH testing in a young onset patient population who had undergone defective DNA mismatch repair (MMR) testing for suspected HNPCC on the basis of young age of onset (< 50 years of age) of CRC. Germline testing within the regions of exon 7 and exon 13 that contain the two most prevalent MYH mutations, Y165C and G382D, was performed on DNA from two hundred and thirty-two individuals with young onset CRC. Biallelic germline mutations were detected in 1.8 % of the MSS young onset CRC cases tested. Monoallelic germline mutations were detected in 1.3 % of those analyzed. These data suggest that MYH testing in young onset CRC even in the absence of multiple colonic polyps may be beneficial in those tumors that have been shown to be MSS.

Interaction of suppressor of fused (Sufu) with the hedgehog (Hh) and Wnt/Wg pathways in Drosophila and mice.

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The Hh pathway plays a key role in embryonic development and in carcinogenesis. Mutations in Ptch, the Hh receptor, are seen in hereditary and sporadic basal cell carcinoma and medulloblastoma. Sufu, like Ptch, down-regulates Hh signaling and is implicated in human predisposition to medulloblastoma. Mammalian cell biochemical studies show that Sufu may also interact with β -catenin, a member of the Wnt/Wg pathway. We performed genetic interaction studies in *Drosophila* and mice to study modifying effects of Sufu on the Hh and Wnt/Wg pathways *in vivo*.

Studies in *Drosophila* have shown genetic interaction between Sufu and Hh pathway members. We bred Ptch +/- mice to Sufu +/- mice to assess possible genetic interaction in mammals. Consistent with previous data, Sufu +/- mice showed no abnormal phenotype, and Ptch +/- mice were significantly larger than Sufu +/- or wild-type mice ($p < 0.02$). Though not statistically significant based on the current number of mice assessed, Sufu/Ptch double heterozygotes tended to be even larger than Ptch +/- mice. No gross external malformations or tumors have been noted in the mice bred to date.

In *Drosophila*, genetic interaction studies between Sufu and Wg pathway members showed that Sufu homozygosity enhanced the wingless phenotype. Apc is a negative regulator of Wnt/Wg signaling through its effect on β -catenin proteolysis. To test for genetic interaction in mammals, Sufu +/- mice were bred to Apc +/- mice. Apc +/- mice, similar to Apc +/- humans, have intestinal polyps, early carcinoma, and reduced lifespan. Segregation analysis of Apc/Sufu cross offspring showed a trend toward reduced viability of Apc/Sufu double heterozygotes compared to Apc or Sufu +/- mice ($p = 0.18$). A strong trend was seen toward increased adult weight of double heterozygotes over Apc +/- mice ($p = 0.06$). Further study will include comparison of gastrointestinal polyp number, size and aggressiveness and analysis of possible extra-intestinal manifestations in these mice.

Ophthalmologic features of patients with DNA repair defects. *B.P. Brooks^{1,2}, C.C. Chan¹, J. Smith¹, J.J. DiGiovanna³, D. Schmidt³, D. Blain¹, K.H. Kraemer³.* 1) NEI, NIH, DHHS, Bethesda, MD; 2) NHGRI, NIH, DHHS, Bethesda, MD; 3) NCI, NIH, DHHS, Bethesda, MD.

Background: Xeroderma pigmentosum (XP) is an autosomal recessive disorder characterized by defective DNA repair mechanisms and an increased risk for ultraviolet (UV) light-induced malignancies on sun-exposed areas such as the skin and conjunctiva. Trichothiodystrophy (TTD) is a related, autosomal recessive disease characterized by sulfur-deficient brittle hair and nails and a broad spectrum of skin, neurologic, immunologic, and developmental abnormalities. TTD patients do not typically develop skin cancer. It is not well-appreciated that many of these patients--including children--suffer from clinically significant ocular surface disease. **Purposes:** 1) To characterize the ocular surface disease in patients with DNA repair disorders. 2) To describe a non-invasive technique for early detection of conjunctival pre-malignancies in these patients. **Methods:** Clinical examination and conjunctival cytology. **Results:** 14 XP and 5 TTD patients underwent age-appropriate, complete ophthalmologic examinations (7 females, 12 males). Schirmer testing for basal tear secretion was reduced (<10mm wetting) in 4 out of 10 XP patients and 3 of 3 TTD patients. Superficial punctate keratopathy was present in 3 of 12 XP patients and 2 of 3 TTD patients. Corneal pannus--ranging in severity from 1mm corneal neovascularization to complete opacification of the cornea--was observed in 5 of 14 XP and 1 of 5 TTD patients. 4 of 5 conjunctival cytology specimens showed evidence of inflammation (neutrophils and lymphocytes); one specimen showed epithelial dysplasia with mitotic figures. **Conclusions:** 1) Ocular surface disease is a frequent clinical finding in patients with DNA repair disorders--including children, in whom ocular surface problems are uncommon. 2) Conjunctival cytology is a non-invasive histologic technique that may be useful in the early detection of conjunctival pre-malignancy.

Characterizing the localization of Rothmund-Thomson syndrome-associated RECQL4. *L.M. Burks, S.E. Plon.*
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Deleterious mutations in *RECQL4*, one of five human RecQ helicase homologues, lead to a significant predisposition to osteosarcoma (OS) in patients with Rothmund-Thomson syndrome (RTS). RTS, an inherited cancer predisposition syndrome, is also characterized by a unique rash and congenital skeletal abnormalities. The regulation and function of *RECQL4* in human cells is not known. Therefore, characterizing the localization of *RECQL4* is critical to understanding its normal role in the cell and OS predisposition in RTS patients. Full-length *RECQL4* cDNA was used to create a GFP fusion construct (*GFP-RECQL4*). Fluorescence microscopy was used to analyze GFP-*RECQL4* in unsynchronized HeLa (cervical carcinoma), WI38VA (SV40-transformed fibroblast), or U2OS (osteosarcoma) cell lines. Localization included cell populations that express GFP-*RECQL4* only in the nucleus, cells that express GFP-*RECQL4* only in the cytoplasm, and cells with both nuclear and cytoplasmic expression. These findings were also confirmed with FLAG-*RECQL4* and are in contrast to the sole published report of exclusively nuclear FLAG-*RECQL4* localization in HeLa cells (Kitao *et al.*, 1999). On average, 10-20% of HeLa or WI38VA cells and 20-50% of U2OS cells express GFP-*RECQL4* only in the cytoplasm. We hypothesize that *RECQL4* localization varies with the cell cycle. By scoring random fields of mitotic cells, identified with antibodies against Aurora B Kinase (ABK) or phosphorylated histone H3 (pH3), we find that cells in telophase (ABK+) are more likely to express cytoplasmic GFP-*RECQL4*. For example, 73% of ABK+ WI38VA cells express cytoplasmic GFP-*RECQL4* compared with 32% of ABK- cells (which may represent cells in early G1). By chi square analysis, the distribution of cytoplasmic cells in the marked and unmarked subsets is non-random ($p < 0.001$). Experiments using pH3 antibodies suggest that GFP-*RECQL4* shifts into the cytoplasm during late G2/prophase. Here, we present evidence that *RECQL4* helicase is expressed outside of the nucleus. Our data suggest that *RECQL4* localization is intrinsically regulated with each cell cycle. We propose that *RECQL4* is excluded from the nucleus during the final stages of mitosis, perhaps to prevent *RECQL4* activity on condensed chromosomal DNA.

Polymorphisms in genes associated with estrogen metabolism, synthesis, binding and penetrance of HNPCC among hMSH2 germline mutation carriers in Newfoundland. *P.T. Campbell¹, M.O. Woods², R. Green², J. Green², W. Fitzgerald³, J.R. McLaughlin¹, H.B. Younghusband².* 1) Epidemiology and Biostatistics, Mount Sinai Hospital, Toronto; 2) Discipline of Genetics, Memorial University of Newfoundland; 3) Sir W. Grenfell Hospital, St. Anthony, NL.

Newfoundland (NL) has the highest provincial incidence and mortality rates of colorectal cancer (CRC) in Canada. The high rates may be partly explained by the strong prevalence of hereditary non-polyposis colorectal cancer (HNPCC) families, uncommon in most populations. Several germline mutations in the mismatch repair gene hMSH2 have been identified in these extended NL families (IVS5+3A>T, deletion of exons 4-16, and an exon 8 deletion). The estimated penetrance of these mutations is high, but not complete, so modifying effects likely exist from other genes or environmental sources. The finding that penetrance is consistently lower in women than men with these genotypes, implies an association with estrogens. Recent work suggests that exogenous estrogens are associated with decreased risk of CRC among women with a strong family history of CRC (and HNPCC). Less understood is the HNPCC risk associated with polymorphisms in genes related with endogenous estrogen levels. Several polymorphisms in genes involved with estrogen synthesis, metabolism and binding have been identified. We postulate that polymorphisms in such genes influence HNPCC penetrance through modification of endogenous hormone levels, including estrogens. We have assembled from the Provincial Medical Genetics Program a collection of samples from 150 confirmed hMSH2 mutation carriers for study of modifier genes associated with HNPCC penetrance, with an additional 50 samples anticipated. In addition, we have collected extensive data on pedigree structure, clinical outcomes (including cancer diagnosis, treatment and death), and we are initiating epidemiologic data collection. The current study will estimate penetrance modification according to allelotype of several common polymorphisms in genes associated with estrogen synthesis (CYP17 and CYP19), estrogen metabolism (COMT) and estrogen ligand binding (ER) among confirmed hMSH2 mutation carriers.

Screening of BRCA1 and BRCA2 mutations in 55 Chilean families: three novel mutations. *M.P. Carvalho¹, L. Rubio¹, C. Torrealba¹, C. Alvarez¹, T. Tapia¹, M. Salinas¹, P. Faundez¹, M. Alvarez².* 1) Dept. Cell and Molecular Biology, Faculty of Biological Sciences, Pontificia Universidad Catolica de Chile, Santiago, Chile; 2) Centro de Cancer, Faculty of Medicine, Pontificia Universidad Catlica de Chile, Santiago, Chile.

Several hundreds of germline mutations at the BRCA1 and BRCA2 genes have been described in families affected of breast and/or ovarian cancer, from worldwide populations. The prevalence of BRCA1 and BRCA2 mutations among affected families is variable, depending on the ethnic origin of the population analyzed. The BRCA1 gene has been mapped to chromosome 17q21 and the BRCA2 gene has been mapped to chromosome 13q12.3. We selected 55 Chilean families by standard criteria, and screened the BRCA1 and BRCA2 genes for mutations, through SSCP, heteroduplex, protein truncation test and sequencing. We found 8 truncation mutations in 11 families. Two families have the 185delAG and 6174delT mutations extensively described in Ashkenazi-Jewish. Three mutations previously described in families from Spain were found in 5/11 Chilean families with mutations. These mutations are: E49X in exon 3 (2 families), 6857delAA, and 5373del GTAT (2 families) in exon 11. Three novel truncation mutations, 308insA in exon 5, 3936C>T, and 4970insTG, were found in other three families. These results reveal that only 20% of Chilean families present mutations on the BRCA1 and BRCA2 genes, and between these 45% show common Spanish mutations. This latter result would be expected considering the important Spanish immigration during the XVI and XVII centuries of colonization. (Fondecyt 1011076).

Family history does not affect survival after breast cancer. *P.O. Chappuis^{1, 2}, H.M. Verkooijen⁴, G. Fioretta⁴, G. Vlastos³, E. Rapiti⁴, C. Bouchardy⁴.* 1) Service of Oncology; 2) Service of Medical Genetics; 3) Service of Gynecology, Geneva University Hospitals; 4) Geneva Cancer Registry, Institute for Social and Preventive Medicine, Geneva University; Geneva, Switzerland.

Background: Family history of breast cancer is associated with an increased risk of developing the disease, but its impact on survival after breast cancer remains uncertain. In a population-based study, we compared survival of breast cancer patients without family history of breast cancer, with that of patients at increased familial risk. **Methods:** Data were extracted from the population-based Geneva familial breast cancer registry, which for each patient includes demographic information, tumour characteristics, type of treatment and survival, and occurrence of cancer among first, second, and third degree relatives. We classified patients into three categories according to the number of family members affected with breast or ovarian cancer, their degree of kinship and age at diagnosis. We calculated breast cancer specific survival and used Cox proportional hazards analysis to estimate the effect of the familial risk on breast cancer survival. **Results:** Between 1990 and 2001, 3709 women were diagnosed with breast cancer. The median follow-up was 7 years. For 3355 (90%) women information on family history was retrieved. Of these women, 2478 (74%) had no relatives with breast or ovarian cancer, 690 (21%) had a moderate familial risk and 187 (6%) a high familial risk. Women with a moderate or high familial risk were significantly younger at diagnosis, had smaller tumours and had a higher incidence of ovarian cancer. Ten year disease-specific survival rates of patients with no, moderate and high familial risk were 79% (95% CI: 76-81%), 82% (95% CI: 77-86%), and 81% (95% CI: 73-89%), respectively. After adjustment for age and stage, there was no difference in breast cancer mortality rates for patients with a positive family history compared to those without an increased familial risk. **Conclusion:** In this large population-based study, a family history of breast or ovarian cancer does not affect survival after breast cancer.

Heterogeneity of *MSH2* rearrangements in HNPCC: an update. F. Charbonnier¹, C. Martin², S. Frérot², S. Olschwang³, Q. Wang⁴, C. Boisson³, MP. Buisine⁵, F. Di Fiore¹, M. Nilbert⁶, A. Lindblom⁷, S. Baert^{1, 2}, T. Frebourg^{1, 2}. 1) Inserm U614, Faculty of Medicine, Rouen, France; 2) Department of Genetics, Rouen University Hospital, 76031 Rouen, France; 3) Inserm U434 and Saint Antoine Hospital, Paris, France; 4) Molecular Oncology, Centre Léon Bérard, Lyon, France; 5) Laboratory of Biochemistry and Molecular Biology, University Hospital, Lille, France; 6) Department of Oncology, University Hospital, Lund, Sweden; 7) Department of Clinical Genetics, Karolinska Hospital, Stockholm, Sweden.

We had previously shown, using QMPSF (Quantitative Multiplex PCR of Short Fluorescent Fragments), that *MSH2* rearrangements are involved in at least 10% of the HNPCC families, fulfilling the Amsterdam criteria. We have identified so far 22 distinct exonic deletions of *MSH2*, removing exon(s) 1, 1-2, 1-4, 1-6, 1-7, 1-8, 1-11, 1-15, 2, 3, 3-11, 4-6, 5, 5-6, 7, 7-10, 8, 9-10, 9-16, 11-14, 12-13, 13-15 and 3 duplications involving exon(s) 7, 7-8, 9-10. We identified only one case of a deletion removing selectively the promoter region. We performed a QMPSF scanning of 200 kb of genomic sequences upstream the *MSH2* transcription initiation site in families with exon 1 deletions. This QMPSF scan revealed at least 13 distinct 5' breakpoints located between 200 kb and 3.6 kb upstream the *MSH2* transcription initiation site. Most of the deletions removed the *TACSTD1* gene encoding the EpCAM adhesion molecule. The QMPSF scan greatly facilitated the location of PCR primers to amplify the rearranged allele and characterize the breakpoints. The characterization of the 5' breakpoints in the recurrent exonic deletions (deletions of exon 1, exons 1-2, 1-6, 1-7, 1-8) showed that these rearrangements had been independently generated. In particular, we identified 6 distinct rearrangements removing exons 1-6, whereas the exons 1-6 deletion had previously been shown to be associated in North America to a founder effect. On the basis of exonic rearrangements and breakpoints within the 5' region, we identified 39 distinct *MSH2* genomic alterations. This study demonstrates the remarkable heterogeneity of the *MSH2* rearrangements and indicates that the *MSH2* region is particularly rich in recombinogenic sequences.

Analysis of the MYH gene mutations Y165C and G383D in familial adenomatous polyposis (FAP) patients negative for APC gene mutations. *B. Chong¹, M.A. Young¹, P. Ward¹, C.S. Richards², B.B. Roa¹, M.R. Hegde¹.* 1) Medical Genetics Laboratories, Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) OHSU DNA Diagnostic Lab Oregon Health & Science University 3181 SW Sam Jackson Park Road, MP-350.

MYH Associated Polyposis is due to autosomal recessive germline mutations in the MYH (MutY) gene, which were identified in ~ 23% of patients with multiple colorectal adenomas. Genetic testing of MYH is indicated for patients wherein: 1) 100 or fewer adenomas are present with or without extracolonic manifestations, particularly if no germ-line APC mutation has been identified, and/or 2) the family history is compatible with autosomal recessive inheritance. The clinical presentation may be consistent with Attenuated Familial Adenomatous Polyposis (AFAP), although only ~10% have identified APC mutations. A small number of patients with apparently classical FAP features were reported to have two mutations in the MYH gene, Y165C and G393D (nomenclature based on MYH transcript NM_012222). These missense mutations have been identified frequently in European Caucasian patients, although various mutations have been identified in different ethnic groups. To estimate the frequency of the Caucasian MYH mutations among patients referred for FAP testing, we analyzed the Y165C and G393D mutations on 129 patients with a clinical diagnosis of FAP and negative APC mutation studies. Analysis was performed using PCR amplification and an automated mini-sequencing method called pyrosequencingTM. We detected mutations in a total of five patients. Two patients were homozygous for the Y165C mutation and one was homozygous for G393D, thereby confirming the diagnosis of MYH-associated polyposis. Two other patients were heterozygous for the Y165C mutation. Thus, the MYH Y165C and G393D mutations were present in approximately 2.3% and 0.8%, respectively, of patient chromosomes in our study. These results suggest that the Y165C and G393D mutations in the MYH gene account for a minority of patients who present with FAP features in a U.S. referral testing population.

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The common deletion 657del5 in the Nijmegen breakage syndrome gene *NBS1* in a pediatric malignant lymphoma/leukemia population a Polish study. *K. Chrzanowska, D. Piekutowska-Abramczuk, E. Popowska, M. Gladkowska-Dura, D. Perek, A. Wakulinska, M. Syczewska, M. Krajewska-Walasek, Polish Pediatric Leukaemia/Lymphoma Group.* Department of Genetics, Children's Memorial Health Institute, Warsaw, Poland.

Nijmegen breakage syndrome (NBS) is a human autosomal recessive disease characterized by genome instability (DNA DSB repair defect) and enhanced cancer predisposition, in particular to lymphoma and leukemia. NBS patients seem to be prevalent among Slavs, especially among the Polish population. All Polish patients identified to date carry a homozygous common mutation, 657del5, within the *NBS1* gene. To examine the role of the 657del5 allele in constitutional susceptibility to childhood lymphoma and leukemia we screened remission DNA samples from 519 children with primary malignant lymphoma and leukemia treated at 12 Hematology and Oncology Centers making up the PPLLSG. All samples were examined for the 657del5 mutation by PCR-SSCP assay. The *NBS1* mutation 657del5 on one allele was present in 5 patients: in 2 of 208 (0.96%) children with non-Hodgkin lymphoma (NHL) and 3 of 248 (1.2%) patients with childhood acute lymphoblastic leukemia (ALL); no mutation was found among 63 patients with Hodgkin lymphoma (HL). Our study showed a 2-fold excess of the major *NBS1* mutation 657del5 among pediatric patients with NHL and ALL as compared with the general population in Poland (0.57%). In addition, 5 new NBS patients were identified within that group. Larger studies are needed to evaluate the impact of the *NBS1* gene heterozygosity in susceptibility to childhood cancers of lymphoid origin.

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DNMT1 participates in GSTP1 promoter region hypermethylation in prostate carcinoma. *D. Chu*^{1, 2}, *C. Wu*¹. 1) Dept of Biomedical technology and laboratory science, & Graduate Institute of Medical Biotechnology, School of Medical Technology, Chang Gung University, Tao-Yuan, 333 Taiwan; 2) Laboratory of molecular diagnosis, Department of Clinical Pathology, Chang Gung memorial hospital, Lin-Kou, Taiwan.

Hypermethylation in the promoter region flanking GSTP1 gene is a common finding in prostate cancer genome. This genetic lesion leads to null expression of Glutathione-S-transferase. In this study, we transduced human DNA (cytosine-5) methyl transferase 1 (DNMT1) into normal prostate cells to investigate whether DNMT1 participated in the methylation status of prostate cell transformation. If so, this methyltransferase could involve in the oncogenesis process of prostate cancer. DNMT1 protein was introduced into non-malignant prostate cells by way of an artificially synthesized peptide carrier and GSTP1 gene promoter region methylation status was determined using methylation sensitive real-time quantitative PCR protocol before DNMT1 treatment and afterward. Data indicated that DNMT1 protein did enter into cell nucleus and it added methyl group onto the cytosine. Therefore, the originally hypomethylated CpG dinucleotides in the GSTP1 promoter region became hypermethylated. Reduced messenger RNA of GSTP1 gene was observed after treatment. In conclusion, we have successfully introduced DNMT1 into the non-malignant prostate cell nucleuses and hypermethylation did occur in these cells. DNMT1 might be an important factor during early stages of prostate neoplastic transformation.

Prevalence of CDKN2A mutations in familial melanoma. *M. Eliason*^{1, 2}, *A. Larson*^{1, 2}, *S.R. Florell*^{1, 2}, *J.J. Zone*^{1, 2}, *L.A. Cannon-Albright*^{1, 2, 3}, *S.A. Leachman*^{1, 2}. 1) Dermatology, University of Utah Health Sciences Center, Salt Lake City, UT; 2) Huntsman Cancer Institute, University of Utah, Salt Lake City, UT; 3) Genetic Epidemiology, University of Utah, Salt Lake City, UT.

Cyclin-dependent kinase inhibitor 2A (CDKN2A) is the major melanoma predisposition gene yet identified. To determine eligibility for genetic testing of melanoma patients, it is essential to establish the prevalence of mutations in subsets of the melanoma population. It has been suggested that pedigrees with two affected members do not warrant testing due to low pre-test probability, whereas pedigrees with more affected members might benefit. Melanoma pedigrees that contain members with pancreatic adenocarcinoma also may have an increased likelihood of carrying a CDKN2A mutation. To determine the prevalence of CDKN2A mutations in subpopulations of hereditary melanoma, 88 pedigrees (n=223) were CDKN2A mutation tested. Pedigrees included 2 or more affected first-degree relatives and were identified through the Utah Population Database. Genetic testing at the CDKN2A locus (p16 and ARF) was performed by sequence analysis of exons 1, 1, 2, 3, promoter region, splice junctions and a deep intronic region. Only 2.5% of pedigrees with two affected members showed a deleterious CDKN2A mutation, whereas 20% of pedigrees with five or more affected members showed a deleterious CDKN2A mutation. An additional 2.5% and 13% of 2-member and >5-member pedigrees had a mutation of uncertain significance, respectively. Of the pedigrees with a deleterious CDKN2A mutation, 50% had a member with pancreatic adenocarcinoma. Four additional pedigrees had member(s) with pancreatic carcinoma, two of these had a CDKN2A polymorphism and two had no CDKN2A mutation. Our data supports the assertion that genetic testing is not warranted for members of pedigrees with two or fewer affected members. Conversely, genetic testing is warranted for pedigrees with 5 or more affected members. Finally, our results demonstrate a strong association between pancreatic cancer and CDKN2A mutations, suggesting that a family history of pancreatic carcinoma should be included as a criteria in consideration of CDKN2A mutation testing.

Hereditary diffuse gastric cancer, cleft lip/palate and *CDH-1/E-cadherin* mutation. T. Frebourg¹, P. Hochain², S. Manouvrier³, S. Lejeune Dumoulin³, JP. Triboulet⁴, M. Scotté⁵, F. Le Pessot⁶, A. Hédouin¹, V. David⁷, M. Blayau⁷. 1) Department of Genetics, Rouen University Hospital, 76031 Rouen, France; 2) Centre Médico-chirurgical du Cèdre, Bois-Guillaume, France; 3) Department of Genetics, Lille University Hospital, France; 4) Department of Surgery, Lille University Hospital, France; 5) Department of Surgery, Rouen University Hospital, France; 6) Department of Pathology, Rouen University Hospital, France; 7) Molecular Genetics Laboratory, Rennes University Hospital, France.

Hereditary diffuse gastric cancer (HDGC), resulting from *CDH-1/E-cadherin* mutations, represents one of the most aggressive cancers, whose medical management is particularly difficult since, in mutation carriers, the normality of endoscopy and biopsies does not exclude the presence of malignant cells. We report a family with a remarkable clinical presentation. The index case, of 23 years of age, was referred for a rectal stenosis and rapidly deceased in the context of a peritoneal carcinosis. The proband's father had developed at the age of 48 a diffuse gastric cancer. A paternal cousin presented at 22 years epigastric pains which led, considering the familial history, to a systematic endoscopy and biopsies revealing a diffuse gastric cancer. *CDH-1* analysis identified a splicing mutation (531 +2 T/A, intron 4) in the index case, his affected cousin and in the proband's paternal uncle, who was asymptomatic at 53 years. This uncle underwent a prophylactic gastrectomy which revealed an hereditary diffuse gastric cancer with metastatic nodes. The proband's brother, aged of 21 years, was asymptomatic and gastroscopy and the biopsies were normal. He underwent a presymptomatic testing who indicated that he was also a carrier. This led to a prophylactic gastrectomy which revealed microscopic foci of intamucosal signet ring cell adenocarcinoma. Remarkably, the index case, his paternal cousin, his paternal uncle and his brother, all affected, had also a cleft lip/palate. This cosegregation suggests that the cleft lip/palate in this family may result from the *CDH-1* mutation. Indeed, mouse models have shown that the E cadherin plays a key role in palatal shelf adhesion during embryogenesis.

BRCA1 and BRCA2 Genetic Variants in A Control Population of 100 African Americans. *C.A. Frye¹, L.A. Burbidge¹, A.E. Deffenbaugh¹, T.M. Scholl¹, S.V. Tavtigian², B.E. Ward¹, W.W. Noll¹.* 1) Myriad Genetic Laboratories, Salt Lake City, UT; 2) International Agency for Research on Cancer, Lyon, France.

Purpose: Determine the prevalence of BRCA1 and BRCA2 sequence variants in a control population of 100 unrelated African Americans. **Background:** Mutations in BRCA1/2 account for the vast majority of cases of hereditary breast and ovarian cancer (HBOC). A significant percentage of the sequence variants identified are variants whose functional significance is not apparent and are classified as variants of uncertain clinical significance. In the U.S. population overall, 12% of the individuals who are tested for BRCA1/2 mutations are found to carry uncertain variants. In the African American population, this figure is more than 3 times higher. **Methods:** One hundred DNA samples from unrelated African Americans were obtained from the NIGMS Repository of the Coriell Institute for Medical Research. Full sequence analyses of BRCA1 and BRCA2 were performed. **Results:** Forty-three different variants were observed, 9 in BRCA1 and 34 in BRCA2. No nonsense or frameshift mutations were detected. In BRCA1 K719E was seen twice, while 8 other variants each were seen once. In BRCA2 I1364L, H2440R and 5UTR218C>T each were seen seven times; K2339N, I2944F and V3244I each were seen six times; D1902N and Q2384K each were seen four times; C1290Y, N1880K and H2116R each were seen three times; P59A, T1414M, D1923A and V3079I each were seen twice and nineteen other variants each were seen once. **Conclusions:** These mutation prevalence data from a control population of 100 unrelated African Americans are an important resource for accurately classifying sequence variants in BRCA1 and BRCA2. Together with prevalence data, cancer cosegregation data and co-occurrence with deleterious mutations in Myriads high risk clinical database, and other published studies, these data will permit the categorization of a large number of BRCA1 and BRCA2 variants, previously classified as variants of uncertain significance, as benign polymorphisms. This will enhance the clinical utility of BRCA1/2 genetic testing for HBOC in African Americans.

Comparison of Chromosomal Radiosensitivity in Breast Cancer Patients and Matched Controls, Using the G2 Assay. *A.L. Georgiou¹, I. Kesterton¹, J. Barwell¹, L. Pangon¹, J. Ball¹, R. Gilchrist¹, R. Camplejohn¹, E. Solomon¹, J. Berg², S. Hodgson¹, Z. Docherty¹.* 1) Guy's and St Thomas' Hospital, London, United Kingdom; 2) Division of Pathology and Neurosciences, University of Dundee, Ninewells Hospital and Medical School.

There is evidence for an inherited component to breast cancer in women without strongly predisposing mutations in BRCA1 and BRCA2, and one hypothesis is that defects in DNA repair and apoptosis may contribute to such susceptibility. It has been shown that patients with Ataxia Telangiectasia, who have a high predisposition to lymphoid malignancies, have an increased chromosomal sensitivity to ionising radiation as demonstrated by increased chromosome and chromatid breaks. This is thought to be due to a defect in DNA repair by homologous recombination. Previous research has suggested that individuals with breast cancer also have an increased chromosomal radiosensitivity in peripheral blood lymphocytes compared with normal controls and may also have a lower apoptotic response. We are looking for evidence of a defect in apoptotic response and impaired DNA repair in individuals who have developed breast cancer.

We have recruited 100 newly diagnosed breast cancer patients prior to treatment and 100 age and sex matched controls. Results of the classical G2 phase chromosome breakage assay are available for the first 64 matched patients and controls and these have been analysed. At present we have found no significant difference in the number of chromosome breaks (in 50 metaphases) caused by ionising radiation in patients compared to controls when lymphocytes were harvested at 30 minutes after irradiation with both half and 1 Grey irradiation. However there is evidence of a reduction in breaks and gaps when counted together in breast cancer patients compared to controls at the 3-hour harvest when irradiated at half Grey. (Paired t-Test, $P = 0.0067$, one tailed). Our results currently do not agree with previous research that has shown a higher radiosensitivity in breast cancer patients. We will also present data on apoptosis in these patients and controls.

Selective disruption of the FANCE/FANCD2 protein interaction: effect on cellular phenotype. *S.M. Gordon^{1,2}, N. Alon¹, M. Buchwald^{1,2}*. 1) Genetics and Genomic Biology, Hospital for Sick Children, Toronto, ON, Canada; 2) Molecular and Medical Genetics, University of Toronto, Toronto, ON.

Fanconi anaemia (FA) is an autosomal recessive disorder characterized by bone marrow failure, cancer predisposition, and increased cellular sensitivity to DNA crosslinking agents. This genetically heterogeneous syndrome consists of at least eleven complementation groups, the genes for eight of which have been cloned. Several of the gene products including FANCA, FANCC, FANCE, FANCF and FANCG participate in a protein complex, the formation of which is required for the monoubiquitination of FANCD2. The direct interaction of FANCE with both FANCC and FANCD2 previously observed in the yeast two-hybrid system has raised the question of whether FANCE can physically link FANCD2 to FANCC and possibly other components of the FA protein complex. The ability of FANCE to mediate an interaction between FANCC and FANCD2 is demonstrated in the yeast three-hybrid system. Immunoprecipitation experiments confirm the association of FANCC with FANCD2 in mammalian cells. The status of this complex in cell lines derived from patients of different FA complementation groups, as well as the possible involvement of the larger FA protein complex, is under investigation. FANCE mutants capable of interacting with FANCC but not with FANCD2 have been identified by yeast two-hybrid screening of a library of GAL4 AD-FANCE constructs randomly mutagenized by passage through an *E. coli* strain deficient in multiple DNA repair pathways. Exogenous expression of these mutants in FA(E) cell lines and subsequent evaluation of DNA crosslinker sensitivity, FA complex formation, and FANCD2 monoubiquitination will be used to evaluate the contribution of loss of the FANCE/FANCD2 interaction to aspects of the FA cellular phenotype. Supported by CIHR and the Hospital for Sick Children Foundation.

Elevated levels of somatic mutation in homozygotes and heterozygotes for inactivating mutations in the FA/BRCA DNA repair pathway. *S.G. Grant¹, S.L. Wenger², W.S. Rubinstein³, J.L. Latimer⁴, W.L. Bigbee⁴, A.D. Auerbach⁵.* 1) Environ Occup Health, Univ Pittsburgh, Pittsburgh, PA; 2) Clin Cytogenet Lab, West Virginia Univ, Morgantown, WV; 3) Center Genet Med, Northwestern Univ, Chicago, IL; 4) Univ Pittsburgh Cancer Inst, Pittsburgh, PA; 5) Lab Hum Genet Hematol, Rockefeller Univ, New York, NY.

Using the blood-based flow cytometric GPA somatic mutation assay we have shown that Fanconi anemia (FA) patients exhibit an ~100-fold increase in allele-loss variants (arising by gene-specific mutations, epigenetic gene inactivation, chromosome loss and deletion). This characteristic has been used in the diagnosis of FA patients; indeed, in this case, the assay can be applied regardless of GPA allelotype. A variable increase in loss-and-duplication variants was also observed, arising by mitotic recombination and chromosome loss and duplication. Neither an increase in this class of variants nor the quantitative increase in allele-loss variant frequency was specifically associated with FA complementation groups A, C and G. FA heterozygotes also exhibited a significant increase in GPA variant frequency ($P = 0.01$). Although only ~2-fold over controls and difficult to use for screening, this increase may be useful for risk assessment. The breast cancer predisposing genes BRCA1 and BRCA2 are functionally associated with the FA pathway, and their cancer susceptibility provides further evidence for a heterozygous FA phenotype. Indeed, BRCA1/2 breast cancer patients have slightly elevated mutation frequencies, both at the autosomal GPA locus (similar to FA heterozygotes) and at the X-linked HPRT locus (not observed in FA heterozygotes). Perhaps most significantly, however, none of these populations, FA homozygotes, heterozygotes or BRCA1/2 carriers exhibit the association between age and somatic mutational burden observed in normal populations. The biological significance of this lack of age effect may be greatest in the more subtly cancer predisposed heterozygous populations, who are at greatest increased risk for mutagenesis and carcinogenesis in early life, when their mutation frequencies are already equivalent to those of aged normal controls.

Geographic Stratification in the Ancestry of Breast Cancer Patients and Carriers of the BRCA2-999del5

Founder Mutation in Iceland. *J. Gudmundsson¹, A. Helgason¹, P. Sulem¹, O. Johannsson², H. Sigurdsson^{2,3}, J. Hrafnkelsson², J. Johannsson², J. Gulcher¹, U. Thorsteinsdottir¹, S. Stacey¹, L. Amundadottir¹, K. Stefansson¹.* 1) Department of Cancer, deCODE Genetics Inc, Reykjavik, Iceland; 2) Department of Oncology, Landspítali-University Hospital, Reykjavik, Iceland; 3) The Icelandic Cancer Center, Landspítali-University Hospital, Reykjavik, Iceland.

Little is known about the geographic stratification of genetic variation and disease risk within most populations. We have cross-referenced data from genetic-, genealogical-, geographical-, and national cancer registry-databases in order to examine this issue on a population-wide scale using breast cancer as an example. We determined the geographical distribution of all Icelandic breast cancer patients diagnosed from 1955-2003. Using the deCODE Genetics population-based genealogy database, we traced the geographical origins of patients' ancestors 5 generations back. These distributions were compared to those of multiple samples of randomly selected matched controls. We detected an excess of breast cancer patients, and their ancestors, in several adjacent counties in the Southwestern part of Iceland. A subset of the breast cancer cohort was tested for presence of the Icelandic founder mutation BRCA2-999del5. Breast cancer patients carrying this mutation were found in strong excess in one of these Southwestern counties. This localization was even more pronounced in the distribution of the carriers' ancestors. This may indicate the point of entry of this mutation into the Icelandic population. Breast cancer patients negative for the BRCA2-999del5 mutation as well as their ancestors showed a geographic localization to the Southwest of Iceland similar to that of all patients. This is consistent with additional genetic risk factors that show an overall geographic bias in Southwest Iceland. Knowledge of geographic locations of individuals, both current and ancestral, can allow for phenotypic stratification of patients that may aid in the genetic dissection of complex diseases.

Surveillance in HNPCC families with frequently occurring urinary tract cancers. *M. Harlan, C. Baeslach, D. Bagley, N. Cardi, B. Boman.* Thomas Jefferson University, Philadelphia, PA.

Introduction: HNPCC has an increased risk of developing cancer: colorectal, endometrial, stomach, and urinary tract. Specifically, individuals with germline mutations in MSH2 have a 10% lifetime risk to develop urinary tract cancer whereas with MLH1 there is a lower but still increased risk. Life-saving treatment is dependent upon early surveillance among organ systems involved. We report treatment and surveillance of urinary tract cancers in 2 families with a significant history of this cancer. Methods: Family 1: The proband presented for genetic counseling due to personal and family histories of cancer. He had bilateral ureteral cancer at 66, his son at 42 and daughter had endometrial cancer at 33. A sister had breast cancer at 70 and renal pelvis cancer at 74. A brother had CRC in his 40s, mother had CRC and endometrial cancer at 47 and her brother had CRC at 47. Family 2: The proband had a right ureteral cancer at 71, cholangiocarcinoma at 69, left ureteral cancer at 67 and endometrial cancer in her 50s. Her father had CRC at 36, sister at 36, 2 nephews at 42 and 35 and son at 44. Her daughter had ovarian and endometrial cancer at 42. Results: Gene sequencing was performed and results were negative for both families. In family 1, the proband's brother subsequently had southern blot analysis which revealed a mutation in the MSH2 gene (deletion of exons 1-8). This mutation would result in truncation of the protein. The proband in family 2 had MSI/IHC which revealed absence of MLH1 expression; further studies have been recommended. Conclusions: Patients with HNPCC have increased risk to develop urinary tract cancer. Often overlooked, it is crucial that gene carriers understand their risks in order to engage in preventive surveillance measures for early detection and optimal treatment. In these families, urinary tract cancer has developed as early as 42. Fortunately, many affected family members can be treated successfully by ureteroscopic laser resection and surveillance has been maintained endoscopically. Surveillance of unaffected members has included cystoscopy and radiographic imaging of the urinary tract in addition to urinalysis and voided cytology.

Involvement of three Finnish *ATM* founder mutations in breast cancer susceptibility. *K. Heikkinen*¹, *J. Tommiska*², *K. Syrjakoski*³, *J. Kere*⁴, *M. Allinen*¹, *S. Karppinen*¹, *K. Rapakko*¹, *K. Holli*⁵, *O. Kallioniemi*³, *H. Nevanlinna*², *R. Winqvist*¹. 1) Dept of Clinical Genetics, Univ of Oulu/Oulu Univ Hospital, Finland; 2) Dept of Obstetrics and Gynecology, Helsinki Univ Central Hospital; 3) Lab of Cancer Genetics, Inst of Medical Technology, Tampere; 4) Dept of Medical Genetics, Univ of Helsinki; 5) Dept of Oncology, Univ of Tampere/Tampere Univ Hospital.

ATM is a major activator cellular response to DNA damage. Loss of both *ATM* alleles results in ataxia telangiectasia (AT), a severe disease exhibiting progressive neuronal degeneration, immunodeficiency, chromosomal instability, radiosensitivity and increased risk of cancer. Although AT is an autosomal recessive disorder, individuals heterozygous for *ATM* mutations have been reported to have some phenotypic effects, including increased risk for female breast cancer. The aim of this study was to confirm the previously suggested association of *ATM* mutations originally identified in Finnish AT families with hereditary susceptibility to breast cancer. Of seven AT related mutations, 6903insA and 7570G>C were the only ones connected to breast cancer. Additionally, a third mutation, 8734A>G, previously associated with breast cancer susceptibility but not reported in AT patients, was observed. In combination with our previous results *ATM* 6903insA, 7570G>C and 8734A>G mutations were observed altogether in 1.4% of the Finnish breast cancer families (9/630) and in 0.5% of the unselected breast cancers (6/1209). All three mutations were absent from healthy controls (0/1307), and the difference in overall mutation frequency between hereditary and unselected cases versus controls was statistically significant ($p < 0.001$ and $p = 0.012$, respectively). In conclusion, our results support the contribution of *ATM* 6903insA, 7570G>C and 8734A>G mutations in hereditary susceptibility to breast cancer. We also provide evidence for founder effects in the geographical distribution of these mutations. The clustering of 6903insA and 8734A>G to the Tampere region seems particularly strong, and together these mutations may regionally contribute to a significant portion of hereditary breast cancer.

A study of cancer incidence in British Fanconi anemia families. *S.V. Hodgson¹, D.F. Easton², J. Ball³, C.G. Mathew³, M.D. Tischkowitz⁴.* 1) Medical Genetics Unit, St Georges Hosp Med Sch, London, London, United Kingdom; 2) Cancer Research UK Genetic Epidemiology Research Group, Strangeways Research Laboratory, Cambridge, United Kingdom; 3) Division of Medical and Molecular Genetics, GKT School of Medicine, Guy's Hospital, London, United Kingdom; 4) Clinical Genetics Unit, Institute of Child Health, London, United Kingdom.

Fanconi anemia (FA) is an autosomal recessive DNA repair disorder with affected individuals having a high risk of developing acute myeloid leukemia and certain solid tumours. Eleven complementation groups have been identified so far and the genes for eight of these are known (FANCA, C, D2, E, F, G, L and BRCA2). Previous studies of cancer incidence in relatives of Fanconi anemia cases have been limited by small sample sizes and have resulted in conflicting results. A study of British FA families was therefore carried out to investigate this question, since increases in cancer risk in FA heterozygotes would have implications for counseling FA family members, and possibly also for the implementation of preventative screening measures in FA heterozygotes. Thirty-six families took part and data was collected on 575 individuals (276 males, 299 females), representing 18,136 person years. In this cohort, 25 males and 30 females were reported with cancer under the age of 85 years and 36 (65%) cancers could be confirmed from death certificates, cancer registries or clinical records. The cancer incidence in a comparable general population cohort was calculated to be 56.95, and the relative risk of cancer was 0.97 (95% C.I.= 0.71-1.23, $p=0.62$) for FA family members. Analysis of relative risk for individual cancer types in each carrier probability group did not reveal any significant differences but confidence intervals were large. Therefore this study, the largest to date, has not shown a significant difference in overall cancer risk in FA families.

Molecular characterization of colorectal cancer in Newfoundland. *A. Hyde¹, M.O. Woods^{1,2}, F. Curtis¹, J.S. Green¹, D. Robb¹, A. Pollett², P.S. Parfrey¹, S. Gallinger¹, B. Bapat², H.B. Younghusband¹.* 1) Discipline of Genetics, Memorial University of Nfld, St. John's, NL, Canada; 2) Dept of Lab Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada.

Newfoundland males have the highest incidence of colorectal cancer (CRC) in Canada and females the third highest. The most common genetic cause of CRC is hereditary non-polyposis colorectal cancer (HNPCC). Alterations in MLH1, MSH2 and MSH6 make up approximately 95% of identified mutations causing HNPCC. The island of Newfoundland has a relatively homogenous population, composed of multiple genetic isolates which have been valuable in genetic research. A population-based study of the Avalon Peninsula of Newfoundland was undertaken over a two year period to recruit all individuals diagnosed with CRC. The purpose of this study was to determine the proportion of hereditary vs. sporadic CRC cases and to determine the genetic basis of disease in hereditary cases. Seventy-nine subjects were recruited, including 22 from high risk families. Tumours from these individuals were retrieved and examined for microsatellite instability, a hallmark of HNPCC, and for immunochemical analyses. Only seven individuals from the high-risk families had microsatellite instability and showed absence of a MMR protein. This indicated there may be other causes for the high incidence of CRC in these families. Therefore, we examined a cohort of 34 high-risk Newfoundland HNPCC-like families which had been ascertained through the Provincial Genetics Program. Automated sequencing of MLH1, MSH2 and MSH6 was used for initial screening of sequence alterations. All tumors also underwent immunohistochemical analyses to determine MLH1, MSH2 and MSH6 expression. Multiplex Ligation-dependent Probe Amplification was undertaken to identify large genomic deletions in MLH1 and MSH2. One mutation, the deletion of exon 8 in MSH2, was identified in five families. Surprisingly, very few of the high risk families screened had identifiable alterations. This could be because these families segregate mutations in unidentified hereditary CRC genes. Future work will include performing a genome-wide scan on such large kindreds.

Linkage analysis of chromosome 8p in the PROGRESS study families: Identification of a locus in older onset hereditary prostate cancer families. *M. Janer¹, J.L. Stanford^{2,3}, K.A. Deutsch¹, D.M. Friedrichsen², S. Kolb³, M. Badzioch⁴, G.P. Jarvik⁴, L. Hood¹, E.A. Ostrander².* 1) Dept Genotyping, Inst Systems Biology, Seattle, WA; 2) Division of Human Biology, Fred Hutchison Cancer Research Center, Seattle, WA; 3) Division of Public Health Sciences, Fred Hutchison Cancer Research Center, Seattle, WA; 4) Department of Medicine, Division of Medical Genetics, University of Washington, Seattle, WA.

Several regions in the genome have been hypothesized to harbor prostate cancer susceptibility genes. Among those, the short arm of chromosome eight appears to be of particular interest. Numerous studies demonstrate that deletions in 8p are among the most common genomic abnormalities observed in prostate cancer tumors. In addition, several family-based linkage studies have also highlighted 8p. In order to investigate the role of genes on 8p in prostate cancer susceptibility within a set of 254 hereditary prostate cancer families (PROGRESS families), we performed nonparametric analysis using markers spanning the chromosome arm at 4cM intervals. Analysis of the entire data set did not yield statistically significant results. However, 111 families with a mean age at diagnosis 66 years demonstrated NPL scores 2 ($p=0.011$) for four consecutive markers spanning about 20cM. The maximal NPL score was 2.42 ($p=0.010$) at marker D8S1989. Multipoint lod scores were negative in this region but lods greater than 2.0 were observed in markers with high NPL scores. Interestingly, the region described by our linkage study overlaps with the minimal deletion interval reported by Swalwell et al. (2002) in a set of sporadic prostate tumors. Several genes encompassed by this region are putative candidates for susceptibility genes. Among them, NKX3.1 is the most interesting as it is expressed at comparatively lower levels in prostate tumors, particularly those associated with advanced stage disease. We are currently investigating this and other candidate genes within the region.

***VHL* germline mutations in Korean patients with von Hippel-Lindau (VHL) disease and pheochromocytomas.**

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von Hippel-Lindau (VHL) disease is an autosomal dominant inherited tumor syndrome characterized by the development of hemangioblastoma; renal cell carcinoma; pheochromocytoma; pancreatic tumors; cysts and cystadenoma in the multiple organs. Pheochromocytomas are catecholamine-secreting tumors that develop from the adrenal medulla. Germline mutations in the *VHL* tumor suppressor gene are responsible for VHL disease. Genotype-phenotype correlations were well established based on the types of mutations in the *VHL* gene. In this study, we investigated for the presence of *VHL* germline mutations in 9 VHL affected patients from 6 families, 1 family with familial pheochromocytoma, and 2 cases of isolated pheochromocytoma or paraganglioma from Korea. Genomic DNA was extracted from peripheral blood lymphocytes, amplified by 6 primer sets spanning all three exons of the *VHL* gene, and sequenced by an automatic sequencer. We found a total of 6 *VHL* germline mutations (5 missense, 1 frameshift), 3 of which were novel types (323_324delGC, P119L, D121N). Three of 6 VHL affected probands had no family history at the time of diagnosis and harbored *VHL* mutations. One family with familial pheochromocytoma had a missense mutation (V84L) previously identified in a family with familial pheochromocytoma from UK. No *VHL* germline mutation was found in 2 cases of isolated pheochromocytoma and paraganglioma. Our results provide more accumulative data for the *VHL* germline mutations and VHL-related phenotypes. To our knowledge, this is the first report of *VHL* germline mutations of Korean patients with VHL disease and pheochromocytomas.

Clinical Testing for Hereditary Paraganglioma. *J.A. Kant¹, J. Uhrmacher¹, B.E. Baysal², F.A. Monzon¹, A.B. Carter¹.* 1) Dept Pathology, Univ Pittsburgh, Pittsburgh, PA; 2) Dept OB-GYN, Univ Pittsburgh, Pittsburgh, PA.

Hereditary paraganglioma (PGL) is an autosomal dominant neoplasia susceptibility syndrome. PGL has been associated with mutations in 3 proteins of the 4-subunit succinate-ubiquinone oxidoreductase (SDH) electron transport complex II - primarily the SDHD and SDHB genes. MRI can be used to follow high-risk mutation-positive patients. Molecular genetic testing for PGL has been offered clinically for several years to ~125 patients from North America with a history of benign or malignant paraganglioma. Bi-directional DNA sequencing of all exons and adjacent intron sequences is performed on the SDHD and/or SDHB genes. The usual reason for testing is to screen probands followed by testing of family members if a mutation is identified. Analysis is occasionally requested on patients with paraganglioma and no obvious family history; there has been one request for prenatal testing. SDHD or SDHB analysis is done after discussion with the submitting professional based on the location of paraganglioma(s) in the proband and family pedigree. SDHD is studied first in those with head and neck tumors and/or a history of paternal transmission. SDHB is studied first when there is maternal transmission and/or abdominal tumors. In the SDHD gene, 5 reported mutations and 6 new variants have been identified including 5 missense, 3 nonsense, 1 frameshift, and 2 splice site changes. In the SDHB gene, 7 reported mutations and 14 new variants have been identified including 11 missense, 3 nonsense, 2 frameshift, and 5 splice site changes. In addition, several polymorphisms have been identified in both genes. 17 probands have shown no mutation in either gene. The clinical significance of new variants is evaluated taking in account predicted effects on the protein, whether disease-associated mutations have been reported at the same amino acid, as well as calculated effects of splice variants on information content. Results are reported following ACMG Recommendations for Interpretation of Sequence Variations. A particularly interesting family without mutation in the SDHD gene demonstrates a haplotype of 3 neutral polymorphic markers which segregate with the paraganglioma phenotype.

A *TP53* truncating germline mutation (R287X) in a family with characteristics of both Hereditary Diffuse Gastric Cancer and Li-Fraumeni Syndrome. I-J. Kim¹, H.C. Kang¹, Y. Shin¹, H-W. Park¹, S-G. Jang¹, S-Y. Han¹, S-K. Lim¹, M.R. Lee², H.J. Chang³, J-L. Ku¹, H-K. Yang², J-G. Park^{1,2,3}. 1) Korean Hereditary Tumor Registry, Cancer Research Institute, Seoul, Korea; 2) Department of Surgery, Seoul National University College of Medicine, Seoul, Korea; 3) Research Institute and Hospital, National Cancer Center, 809 Madu-dong, Ilsan-gu, Gyeonggi, 411-764, Korea.

Mutations in *CDHI* have been associated with hereditary diffuse gastric cancer (HDGC) in Western populations, but have not been shown to play a major role in Asians. Recently, a patient with familial gastric cancer (FGC) was shown to harbor a germline mutation in the *TP53* gene, which encodes TP53 and has been previously associated with Li-Fraumeni Syndrome (LFS). To determine whether mutations in *TP53* are associated with familial gastric cancer (FGC) in Asians, we screened the entire coding region of *TP53* in probands from 23 Korean FGC families by an automatic sequencing or DHPLC (denaturing high performance liquid chromatography). We identified a nonsense (R287X) *TP53* germline mutation in a family whose history is compatible with both HDGC and LFS. Two members of this family (SNU-G2) were afflicted with brain tumors, seven with gastric cancers, two with sarcomas and one with both gastric cancer and a sarcoma. It is possible that the HDGC/LFS phenotype in family SNU-G2 family may be due to an aggressive nonsense mutation (R287X) in exon 8. The R287X *TP53* mutation segregated with the cancer phenotype in the family members from whom DNA samples were available. Immunohistochemical staining for p53 did not show any p53 nuclear protein accumulation in the tumor cells of the liposarcoma or medulloblastoma samples. Although a *TP53* germline missense mutation was previously reported in a case of LFS presenting with a non-familial gastric cancer, this is the first report of a large family with a *TP53* germline mutation associated with both HDGC and LFS. Our result suggests that researchers should be cautious in screening familial gastric cancer families for mutations in *TP53*, as *TP53* mutation-carrying FGC families may also develop LFS-related phenotypes.

Detection of a FANCA SNP haplotype associated with early onset breast cancer. *C. Lazaro*^{1,2}, *Q. Wang*¹, *S. Shah*¹, *Y. Chang*¹, *L. Moreau*³, *A. d'Andrea*³, *D. Bell*¹, *D. Haber*¹, *M. Daley*⁴, *A. Bernards*¹. 1) MGH Center for Cancer Research, Charlestown MA; 2) Medical & Molecular Gen. Cent., IRO, Barcelona, Spain; 3) Pediatric Oncology, Dana-Farber Cancer Institute, Boston, MA; 4) Whitehead Institute for Biomedical Research, Cambridge, MA.

Breast cancer (BC) is the most common type of cancer among women, with an estimated 1 million new cases annually. Having a first degree affected relative is among the most important risk factors for BC, but mutations in known tumor suppressors such as BRCA1 and BRCA2 account for only a small proportion of non-familial cases. It has been suggested that a polygenic model with multiple predisposing alleles, each with a small effect on disease susceptibility, may underlie the 75% unexplained familial cases and the majority of sporadic cases. In an attempt to identify new BC susceptibility genes, we analyzed missense SNPs of candidate genes in a case-control association study. For this project we assembled a database of >300 genes that may be involved in maintaining genome stability. We identified 964 missense SNPs in these genes, including 176 that have a minor allele frequency >0.04. Given the functional links between BRCA2 and Fanconi Anemia (FA) genes, we surveyed missense allele genotypes among FA genes in an exploration panel consisting of ~400 early onset (age at diagnosis <40 years) patients and ~220 controls. Positive findings were confirmed in an independent 200 patient/200 control validation panel. All but three FA genes do not harbor common missense SNPs. Common missense SNPs in FANCD2 and FANCL showed no altered genotype distributions among cases and controls, but several FANCA missense SNPs were strongly associated ($p < 0.001$) with early onset BC. Genotyping of 11 FANCA SNPs and 2 SNPs in the adjacent CDK10 gene allowed the identification of a disease-associated SNP haplotype. Additional studies will need to confirm these findings and assess to what extent the associated haplotype contributes to BC susceptibility.

Barriers to cancer surveillance in women at risk for Hereditary Breast and Ovarian Cancer. *M. Lilley¹, J. Liu¹, D. Gilchrist², D.C. Dover¹, C. King¹, K. Barker³, R. Hughes³.* 1) Alberta Cancer Board, Edmonton, Canada; 2) University of Alberta, Edmonton, Canada; 3) University of Calgary, Calgary, Canada.

OBJECTIVE: This follow-up study examined factors associated with cancer surveillance behaviour. In our previous study of 171 women who had genetic counseling for Hereditary Breast and Ovarian Cancer (HBOC), but with no personal history of breast or ovarian cancer, only 60% of women had annual mammograms, 18% had annual ovarian ultrasounds and 15% annual CA125 blood tests. **METHOD:** Questionnaires were sent to an initial sample of 77 women. To date, 51% of those questionnaires have been returned. **RESULTS:** Only 89% of women believe that they are at higher risk for developing breast cancer than the general population. Sixty-nine percent of this perceived at-risk group had annual mammograms. Fifty-six percent believe that they are at higher risk for developing ovarian cancer than the general population, with only 42% having annual ovarian ultrasounds, and 21% annual CA125 testing. Seventy-seven percent had discussed mammography with their MDs but only 63% of this group had annual mammograms. Fifty-nine percent discussed ovarian ultrasound with their MDs but only 45% of this group had annual ultrasounds. Forty-four percent discussed CA125 tests with their MDs but only 40% of this group had annual blood tests. Eighty-four percent reported that they were encouraged by their MDs to have mammograms. Fifty-six percent and 33% reported being encouraged by their MDs to have ovarian ultrasound, and CA125, respectively. Of the participants who felt encouraged by their MDs, 69% had annual mammograms, 47% had ovarian ultrasounds and 50% had CA125. Of the participants who did not feel encouraged by their MDs, none had annual mammograms, ovarian ultrasounds or CA125. **CONCLUSION:** Women who have received genetic counselling for HBOC do not necessarily believe they are at increased risk for developing breast and ovarian cancer. Among those who acknowledged their increased cancer risk, utilization of ovarian cancer surveillance tests was lower than mammography. Compliance with the cancer surveillance recommendations was greater for women who felt encouraged by their MDs than those who did not.

Clinical manifestation of non-hereditary retinoblastoma is influenced by a parent-of-origin effect in a subset of patients. *D. Lohmann*¹, *A. Schüler*², *C. Jurklies*², *M. Neuhäuser*³, *T. Lehnert*⁴, *S. Weber*⁴. 1) Humangenetik; 2) Augenklinik; 3) IMIBE; 4) Klinische Forschergruppe Universitätsklinikum Essen, Germany.

Mutation analysis is part of routine management in patients with isolated unilateral retinoblastoma (RB). To date, we have identified both oncogenic RB1 gene mutations in tumors from 230 of 263 of patients (87%). In 34 patients (13%), a mutation was also detected in constitutional DNA (peripheral blood), including 14 with obvious mutational mosaicism. To identify any differences in phenotypic expression between patients with and without a constitutional mutation we adopted a data warehouse approach to link up all relevant data including clinical data, histopathology, and genetic findings. Data were extracted from source systems (patient oriented databases), transformed, and loaded into a data mart using business intelligence software (COGNOS). Datasets complete with all relevant data on phenotypic expression were available from 237 of 263 patients. Among other findings we found that the distribution of age at diagnosis in patients with a constitutional mutation was not significantly distinct from that of patients without a constitutional mutation. This contrasts the findings in many hereditary cancers, where age at diagnosis is earlier compared to that of sporadic disease. To identify factors associated with early diagnosis we compared distributions grouped by certain attributes (drilling down). Overall, multifocal RBs were diagnosed earlier than unifocal RBs. However, in several patients with multifocal RB both oncogenic mutations were not detected in blood DNA. The distributions of age at diagnosis of tumors with different RB1 mutations were not distinct. Analysis of age at diagnosis of tumors with LOH showed that several patients with non-hereditary RB and retention of the paternal allele were diagnosed early. This caused an extra peak early in the distribution that was absent in non-hereditary patients with retention of maternal alleles. Our findings suggest that in a subset of patients with non-hereditary RB age at diagnosis of is influenced by a parent-of-origin effect.

A germline intronic mutation in *CDKN2A* affects splicing and predisposes to melanoma. *J.C.Y. Loo*¹, *L. Liu*², *L. Gao*², *A. Hao*², *M. Duvic*³, *D. Hogg*^{1,2,4}. 1) Medical Biophysics, University of Toronto, Toronto, ON, Canada; 2) Clinical Sciences, University of Toronto, Toronto, ON, Canada; 3) Dermatology, M.D. Anderson Cancer Center, Houston, TX; 4) Medicine, University of Toronto, Toronto, ON, Canada.

Cutaneous malignant melanoma (CMM) occurs primarily in European-derived populations. In North America, the lifetime incidence approximates 1/100, and the mortality rate per case is over 2 per 100,000. A melanoma in its early stages can be cured by surgical excision; however, deep melanomas tend to metastasize and are typically unresponsive to traditional therapies. Surveillance of CMM-prone individuals and populations should decrease the morbidity and mortality associated with this disease.

The *CDKN2A* gene at the chromosomal locus *9p21* encodes the p16^{INK4a} and p14^{ARF} cell cycle regulators. Coding mutations of this gene cosegregate in approximately 25% of melanoma families, but the genetic alterations in a subset of *9p21*-linked kindreds remain unknown. One such family, who contributed to the initial mapping of the melanoma susceptibility gene in 1994, has been afflicted with melanomas and multiple primary melanomas for at least five generations. We sequenced all *CDKN2A* exons and the adjacent intronic regions from the genomic DNA of affected family members, but found no coding mutations. RT-PCR of the p16^{INK4a} and p14^{ARF} transcripts each yielded two products: one of expected-sized and one larger product. Sequence analyses of individual RT-PCR products revealed that both aberrant transcripts contain 196 bp of intron 2. Examination of intron 2 at the genomic DNA level uncovered an IVS2-284A>G mutation that predicts a consensus splice acceptor site. Additional splicing of the RNA occurs between a cryptic splice donor site 196 bp downstream of the mutation and the Exon 3 splice acceptor. The IVS2-284A>G mutation cosegregates with melanoma cases within the family. Similar non-coding mutations may account for additional mutation-negative families, thus allowing the detection of additional at-risk kindreds.

A possible causative role for nonhomologous end-joining DNA repair in the occurrence of a complex deletion in

MLH1. S. McVety¹, R. Younan², N. Wong^{1, 3, 5}, G. Chong^{1, 3, 4}, W.D. Foulkes^{1, 3, 4, 5}. 1) Human Genetics, McGill University, Montreal, Canada; 2) Surgery, McGill University, Mtl, Can; 3) Cancer Prevention Centre, SMBD Jewish General Hospital, McGill University, Mtl, Can; 4) Diagnostic Medicine, SMBD Jewish General Hospital, McGill University, Mtl, Can; 5) Genetics, SMBD Jewish General Hospital, McGill University, Mtl, Can.

Hereditary non-polyposis colorectal cancer (HNPCC) is an inherited cancer syndrome caused by a defect in the mismatch repair pathway. The majority of HNPCC mutations have been detected in *MLH1* and *MSH2*. Most reported mutations are substitutions, small insertions and deletions, but standard methods of mutation analysis do not detect large rearrangements. It is now established that large deletions, insertions and rearrangements account for a significant proportion of *MLH1* and *MSH2* mutations.

Large deletions in *MLH1* are frequently attributed to homologous recombination between *Alu* repeats, since *Alu* sequences are present at or near most deletion breakpoints. *MLH1* contains an average of one *Alu* for every 1.5 kb of sequence and it has been suggested that a high density of *Alu* repeats may promote recombination. However, the presence of *Alu* sequences does not *per se* increase the frequency of large deletions. The fact that *Alu* sequences are found near deletion breakpoints in *MLH1* may simply be due to the high frequency of *Alu* in the gene.

Here, we report an unusual rearrangement resulting in the deletion of exons 6, 7 and 8 of *MLH1*, with the retention of part of intron 6 and insertion of two nucleotides at each end of the retained sequence. The 349 bp retained sequence is made up of two closely spaced *Alu* sequences. A class of deletions coupled with the insertion of an inverted sequence derived from the deleted region has previously been identified. Our case is unique in that the retained sequence is not inverted. There is no homology between the deletion breakpoints or insertion junctions and the insertion of base pairs is typical of repair mediated by nonhomologous end-joining (NHEJ). We propose a mechanism involving NHEJ to explain the occurrence of this complex deletion.

Microsatellite Instability Analysis In Colorectal Cancer Patients From Colombia: A Novel C399T Polimorphism In MSH2 Gene. *Y. Montenegro¹, J.L. Ramirez-Castro¹, G. Bedoya², L.F. Isaza^{1,3}, F. Quintero-Rivera^{1,4}, C.M. Muneton-Pena¹.* 1) Medical Genetic Unit; 2) Molecular Genetic Group; 3) Department of Surgery, School of Medicine, University of Antioquia, Hospital Universitario San Vicente de Paul, Medellin, Colombia; 4) Center for Human Genetic Research, Massachusetts General Hospital, HMS, Boston, MA.

Colorectal cancer (CRC) is one of the most common forms of cancer in humans. The familial cancer syndrome known as hereditary nonpolyposis colorectal cancer (HNPCC) is the most common hereditary colon cancer syndrome. HNPCC has been associated with germline mutations in several DNA mismatch repair genes such as MLH1, MSH2, MSH6, PMS1 and PMS2. Mutations of these genes cause microsatellite instability (MSI). MSI accelerates the carcinogenic process through an increase in the mutation rate. Our aim was to analyse colorectal cancer patients for MSI and to do mutation analysis in positive MSI patients for MLH1 and MSH2. A total of 41 patients were analysed using six markers (BAT-25, BAT-26, BAT-40, D17S250, D2S123 and D5S346) and four neutral STR. Mutation in MLH1 and MSH2 genes was examined in the MSI positive patients using direct sequencing with Genetic Analyzer. Eleven out of the 41 patients with CRC were diagnosed with HNPCC. MSI was detected in 14/41 (34.1%) of the tumors. MSI was found to be high (MSI-H) in 11/14 (75.6%) tumors and three (14.4%) were low (MSI-L). No mutations for MLH1 and MSH2 genes were detected in 11 of the MSI-H cases that had HNPCC. A novel polymorphism in exon 3, C339T, at codon 133 (GATGACASP) was detected. This polymorphism was also found in sporadic CRC patients and in healthy individuals. The level of MSI found was almost identical to the previously reported in HNPCC. Our analysis does not exclude mutations in other mismatch repair genes (i.e. MSH6). Additional mutations could also be responsible for HNPCC in our patients. It is also possible that an unusual abnormality in either the MLH1 or MSH2 genes, which can not be identified by the current genetic testing, be responsible for the HNPCC in these patients. Therefore further analysis is required in order to determine the role of this polymorphism in Colombian CRC families.

Population-based characterization of HNPCC - an unbiased analysis of cancer prevalence. *D.W. Neklason¹, G. Mineau¹, W. Samowitz², R. Kerber¹, J. Barlow¹, H. Hanson¹, R.W. Burt¹.* 1) Huntsman Cancer Inst, Univ Utah, Salt Lake City, UT; 2) Department of Pathology, Univ Utah, Salt Lake City, UT.

Hereditary Nonpolyposis Colon Cancer (HNPCC) is cancer syndrome caused by mutations in the DNA mismatch repair genes, primarily MLH1 and MSH2, and hallmarked by different cancers (principally colon and uterine), incomplete penetrance, and an early age of cancer diagnosis. Current estimated cancer risks for HNPCC have been developed mainly from families seeking medical advice. This can lead to biased estimates due to the small family size and a lack of communication or knowledge within families about cancer diagnoses. The objective of this study is to examine pedigrees of HNPCC probands using the Utah Population Database (UPDB), which includes genealogies linked to cancer records from the Utah Cancer Registry, and Utah death and birth certificates. This gives an unbiased perspective by including all cancers reported in the state of Utah back to 1967. Microsatellite instability (MSI) was examined in 383 high-risk colon cancers (diagnosed under age 50, or two first-degree relatives with colon cancer). A combination of family history, age of onset, and immunohistochemistry and sequencing of MLH1 and MSH2 were used to identify likely HNPCC cancer cases from 51 MSI-positive cancers. Family history was available in UPDB (two generations or more) on 36 cases with some kindreds going back 8 generations. Eleven cases (8 kindreds) fulfill Amsterdam II criteria, however 17 cases (14 kindreds) are likely to be HNPCC based on combined molecular results. A detailed analysis of cancer prevalence from the multiple HNPCC families will be presented. In one MSH2 kindred of 6 generations, HNPCC-associated cancers were traced in descendants of 3 of 9 branches. A total of 27 HNPCC-associated cancers were identified in 20 people including 14 colorectal cancers, 5 uterine cancers, and 6 cancers of the urinary tract at average ages of 58.8, 56.4 and 52.5 years respectively. The large number of urinary tract cancers was a surprise as current risk to mutation carriers is estimated at only 4% by age 70. Mutation testing will be required in family members for precise cancer penetrance estimates.

A Retrospective Family Study of Medulloblastoma. *D. Ng¹, T. Stavrou^{1, 2}, L. Liu³, M.D. Taylor⁴, B. Gold⁵, M. Dean⁵, H.S. Nicholson², J. Byrne², J.T. Rutka⁴, D. Hogg³, G.H. Reaman², A.M. Goldstein¹.* 1) Genetic Epidemiology Branch, DCEG, NCI, National Institutes of Health, DHHS, Bethesda, MD, USA; 2) Children's National Medical Center, Washington, DC, USA; 3) University of Toronto, Toronto, Canada; 4) Hospital for Sick Children, Toronto, Canada; 5) Center for Cancer Research, NCI, National Institutes of Health, DHHS, Frederick, MD, USA.

Medulloblastoma is the most common malignant central nervous system tumor of childhood and can occur sporadically or in association with inherited cancer susceptibility disorders such as the nevoid basal cell carcinoma syndrome (NBCCS). To determine whether an association existed between medulloblastoma and as yet undefined genetic syndromes we retrospectively reviewed clinical data on 33 patients with medulloblastoma from Children's National Medical Center (CNMC) diagnosed from 1969-1997 and compared them with their unaffected relatives (n=46). Among this study group, six had tumors showing desmoplastic histology. Two of the six met the diagnostic criteria of NBCCS. One NBCCS patient had a missense mutation of patched-1 (PTCH1); the other had no identifiable PTCH1 mutation. Two patients with isolated desmoplastic medulloblastoma were found to have an insertion and splice site mutation respectively in SUFU (suppressor of fused). Two remaining patients with desmoplastic medulloblastoma had no identifiable mutations by sequencing in PTCH1 or SUFU. All patients with nondesmoplastic medulloblastoma histology received molecular testing for SUFU. A subset (n=5) received mutation screening of PTCH1. None of these patients had an identifiable mutation in PTCH1 or SUFU. Clinical analysis of the 31 medulloblastoma patients without NBCCS revealed an elevated frequency of short stature ($p<0.01$) and shortened lower segment ($p<0.01$) compared with unaffected parents. This was not an unexpected finding since cranial spinal radiation therapy, a common treatment therapy for medulloblastoma, is associated with short stature. There was a paucity of clinical findings among the majority of medulloblastoma patients in this sample to suggest a definable cancer genetic syndrome. Thus, the etiology of sporadic nondesmoplastic medulloblastoma remains undefined.

Do germline monoallelic *MYH* and *MSH6* missense mutations act together in cancer development? R.C. Niessen¹, R.H. Sijmons¹, J. Osinga¹, M. Ligtenberg², F.B.L. Hogervorst³, C.H.C.M. Buys¹, J.H. Kleibeuker⁴, R.M.W. Hofstra¹. 1) Department of Clinical Genetics, University Hospital Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands; 2) Department of Human Genetics, University Medical Centre, Nijmegen, The Netherlands; 3) Netherlands Cancer Institute, Amsterdam, The Netherlands; 4) Department of Gastroenterology, University Hospital Groningen, Groningen, The Netherlands.

The MYH protein, involved in the repair of oxidative DNA damage, interacts with the MMR protein MSH6, thus implying an interaction between the DNA base excision and DNA mismatch repair (MMR) systems. Therefore, it is conceivable that germline mutations of *MYH* and of a MMR gene, in particular *MSH6*, in the same individual may result in an increased cancer risk. We postulate that a heterozygous *MYH* mutation might contribute to cancer susceptibility in carriers of a MMR gene (in particular *MSH6*) mutation. This might be most apparent in the case of missense mutations, as these might result in a less severely impaired MMR system, rather than in cases of truncating mutations. We have studied the frequency of germline *MYH* mutations in colorectal and/or endometrial cancer patients with proven germline missense or truncating mutations of the MMR genes *MLH1*, *MSH2* and *MSH6*. The frequency of monoallelic *MYH* mutations in the Dutch general population is 2%. Among 40 patients carrying a germline missense MMR gene mutation we identified 4 carriers of a monoallelic *MYH* mutation. Among 41 patients with a truncating MMR gene mutation we identified 1 monoallelic *MYH* mutation. More specifically, we identified 3 monoallelic *MYH* mutations in 14 *MSH6* missense mutation carriers (21%, $P < 0.05$ compared with the normal Dutch population), 1 in 7 *MSH2* missense mutation carriers (14%, n.s.) and 1 in 22 *MSH2* truncating mutation carriers (5%, n.s.). No *MYH* mutations were observed in 19 *MLH1* missense mutation carriers, 14 *MLH1* truncating mutation carriers and 5 *MSH6* truncating mutation carriers. Our preliminary results suggest that monoallelic *MYH* mutations in combination with a missense MMR gene mutation, in particular in *MSH6*, may indeed lead to an increased risk to cancer development.

REV3 and RAD51 function in pathways separate from Fanconi anemia for interstrand crosslink repair. *S.B. Olson, A. Hemphill, Y. Akkari, Y. Torimaru, A. Hanlon Newell, R.E. Moses.* Department of Molecular and Medical Genetics, Oregon Health & Sci Univ, Portland, OR.

Fanconi anemia is an autosomal recessive disease characterized by genomic instability, manifest as decreased survival and chromosomal breaks and radial formation on exposure to DNA interstrand crosslinks (ICLs). Findings suggest the involvement of additional DNA repair pathways in ICL repair. This is indeed the case in *Saccharomyces cerevisiae* in which repair of ICLs involves three separate pathways, represented by *REV3*, *RAD51* and *SNMI*. *REV3* protein acts in the post-replication repair (PRR) pathway, and is the catalytic subunit of DNA polymerase zeta, a translesion bypass polymerase. *RAD51* protein is required for homologous recombination (HR). In order to assess the roles of *REV3* and *RAD51* in ICL repair in human fibroblasts, and their relation to the FA pathway, we have used siRNA to deplete the proteins and measured the response to mitomycin C (MMC), an ICL agent. Depletion of either *REV3* or *RAD51* produces a significant increase in cell death and radial formation in normal fibroblasts with MMC treatment. Depletion of *REV3* or *RAD51* in a FANCA cell line produces an added increase in sensitivity and radial formation, indicating a non-epistatic relationship with the FA pathway. When the FA protein *BRCA2* was depleted simultaneously with *RAD51*, there was no increase in sensitivity above that of *RAD51* depletion alone. In contrast, simultaneous depletion of *REV3* and *RAD51* produced an increased cellular sensitivity and radial formation compared to individual depletion. Our findings indicate that *REV3*, *RAD51* and FANCA proteins represent three separate pathways for repair of ICLs, with *RAD51* acting in the FA pathway as well as at least one additional pathway. We also conclude that *RAD51* and *REV3* are not required for radial formation in human cells, indicating that neither bypass or HR functions are needed.

Frequency of founder BRCA mutations in French Canadian breast/ovarian cancer families and identification of a new founder BRCA2 mutation. *K. Oros¹, P. Ghadirian², A.M. Mes-Masson³, W.D. Foulkes¹, D. Provencher³, P. Tonin¹.* 1) Human Genetics, McGill U, Montreal, PQ; 2) Epidemiol Res Unit CR-CHUM (Htel Dieu), Montreal, PQ; 3) CR-CHUM (Hpital Notre-Dame)/Institut du cancer de Montral, Montreal, PQ.

Specific recurrent BRCA1 and BRCA2 mutations in hereditary breast cancer (HBC) and breast-ovarian cancer (HBOC) families of French Canadian (FC) descent, attributed to common founders, contribute to a significant proportion of mutation positive cancer families. We have extended this study of the contribution of the common mutations to a larger cohort of high-risk FC cancer families and included new mutations reported in FC cancer families. The 241 FC families (149 HBC and 92 HBOC) contained at least 3 cases of BC (<65) and/or OC in 1st, 2nd or 3rd degree relatives of an index case affected with BC/OC. DNA from an index case was analyzed for the 3 BRCA1 and 4 BRCA2 mutations previously shown to recur in FC cancer families and 8 BRCA1 and 5 BRCA2 mutations recently identified in FC cancer families. Mutations were identified in 99 (41%) families: 46 BRCA1-positive and 53 BRCA2-positive. There were 53 mutation-positive HBOC families and 46 mutation-positive HBC families. A significant proportion (73%) of mutation positive families harbored a previously reported recurrent mutation (4446C>T, 2953del3insC, 6085G>T and 8765delAG). Seven families harbored the BRCA2 mutation, 3398del5. This mutation was also identified independently in two families from Hereditary Cancer Clinics of Montreal. To assess if carriers of this mutation share a common ancestry, the FC families harboring this mutation were analyzed using microsatellite markers flanking BRCA2. Haplotype analyses suggested that carriers of this mutation share a common ancestry. Taken together, these findings suggest that while FC cancer families are more genetically heterogeneous than previously demonstrated, the recurrent mutations previously identified in FC cancer families remain the most common mutations identified. A mutation screen of FC families that also includes the newly discovered recurrent mutation would account for 80% of mutation positive FC cancer families.

A Preliminary Study on Cytochrome P450 1A1 Gene Polymorphisms as Possible Biomarkers of Cancer Risk Among Filipinos. *C.D. Padilla¹, E.C. Cutiongco¹, C.L.T. Silao¹, C. Ngelangel²*, *Philippine Cancer Genetics Research Group.* 1) Institute of Human Genetics, National Institutes of Health Philippines, University of the Philippines Manila; 2) Oncology Study Group, Institute of Clinical Epidemiology, National Institutes of Health Philippines, University of the Philippines Manila and Section of Medical Oncology, Department of Medicine, Philippine General Hospital and Jose R. Reyes Memorial Medical Center.

Population-based studies on the genetic variations of metabolizing enzymes have shown associations between polymorphic genes and increased susceptibility to some types of cancer. This preliminary report looks into the associations between susceptibility to lung and oral cavity cancer and specific genetic polymorphisms involving the Phase I metabolizing enzyme CYP1A1. Demographics, medical/social/diet histories and occupational exposure data are also presented. To identify these polymorphisms, polymerase chain reactions (PCR) and restriction enzyme polymorphism studies have been employed on DNA obtained from 500 blood samples of Filipino cancer patients and normal controls. Results show that mutant types for CYP1A1 MspI and NcoI suggest protective effects therefore decreased susceptibility to lung cancer. CYP1A1 MspI mutant genotype also suggest protective effects for oral cavity cancer. For the CYP1A1 MspI polymorphism, smoking tends to significantly raise lung cancer risk among those with mutant and heterozygous genotypes while not for those with wild genotypes. The reverse is true of the effects of smoking to oral cancer susceptibility. CYP1A1 NcoI wild genotype susceptibilities to both lung and oral cavity cancers increase with smoking. Further studies using more subjects are needed to determine whether these data are statistically significant.

Turcot syndrome in a family with hMLH1 mutation. *J.G. Pappas¹, T.P. Nicolaidis², E. Reich¹.* 1) Clinical Genetic Services, New York Univ, Sch of Med, New York, NY; 2) Department of Pediatrics, New York University, Sch of Med, New York, NY.

We present a family with multiple members affected with hereditary nonpolyposis colorectal cancer (HNPCC) and glioblastoma (GBM). The proband is a 9 year old boy with glioblastoma multiforme. His father had non polyposis colon cancer diagnosed at 34 year of age. Five generation pedigree revealed that twelve members of the family were diagnosed with early onset colon cancer and three with GBM including the proband. The family fulfills the Amsterdam criteria for HNPCC. Turcot syndrome is the association of colorectal cancer or colorectal polyposis with a primary tumor of the intracranial central nervous system. Turcot syndrome in families with familial adenomatous polyposis and medulloblastoma is associated with mutation in the APC gene. Mutations in the genes associated with HNPCC (hMLH1, hMSH2) are reported in the medical literature in families with Turcot syndrome that the brain tumor is GBM. DNA testing of both the patient and the patients father revealed a missense mutation (position 1731 G>A) of amino acid 577 in the last nucleotide of exon 15 of the hMLH1 gene. This mutation is associated with splicing errors and leads to an out of frame deletion of exon 15 (Wijnen et al., 1996). Further evidence that this deletion was pathogenic was provided by immunohistochemistry on the patients tumor. An immunoperoxidase stain performed with an antibody directed toward DNA mismatch repair enzyme MLH-1 showed aberrant loss of expression in the malignant cells with intact positive control expression in the benign background cells. The missense mutation in hMSH1 in this family with Turcots syndrome has been previously described to cause HNPCC but not Turcots syndrome. The only other mutation in hMLH1 associated with Turcot syndrome and previously described in the medical literature was a three nucleotide deletion (Hamilton et al, 1995). This family further establishes the association of HNPCC and GBM (Turcot syndrome) with mutations in hMLH1.

Replication protein A associates with double-strand DNA breaks in an ATM and DNA-PKcs dependent manner.

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Replication protein A (RPA) is a heterotrimeric single-strand DNA-binding protein involved in DNA replication, recombination, and repair. RPA is one of many proteins phosphorylated in response to ionizing radiation (IR) by the protein kinases ATM and DNA-PKcs. IR-induced phosphorylation modulates RPA's ability to support DNA replication *in vitro* and is thought to re-direct RPA from DNA replication to DNA repair. To better understand the effects of IR-induced RPA phosphorylation on RPA's biological functions, we are studying the effect of ATM and/or DNA-PKcs deficiency on RPA's participation in the repair of double-strand DNA breaks (DSBs).

Using short hairpin RNAs (shRNAs), we have achieved stable, effective, and specific silencing of DNA-PKcs expression in wild type and ATM-deficient human fibroblasts. Silencing is detectable by both Western analysis and immunofluorescence. Silenced cells display increased sensitivity to IR, as predicted from the phenotype of DNA-PKcs mice. Our creation of human cells deficient in both DNA-PKcs and ATM is the first of this kind; a previous study reported synthetic lethality between mutations in ATM and DNA-PKcs in mice.

Using laser micro-beam irradiation, we find that RPA localizes to photo-induced DSBs in wild-type human fibroblasts. In contrast, RPA does not associate with laser-induced DSBs in cells deficient in either ATM or DNA-PKcs, or in cells lacking both kinases. This is the first report of an effect of ATM and/or DNA-PKcs-deficiency on an *in vivo* function of RPA. In order to further elucidate the role of phosphorylation in recruiting RPA to induced DSBs, we now are determining the kinetics of RPA association with induced DSBs in cells depleted for ATM and/or DNA-PKcs, as well as studying the behavior of non-phosphorylatable and constitutively phosphorylated RPA mutants.

Prevalence of Y165C and G382D germline mutations of the *MYH* gene in Canadian Familial Adenomatous Polyposis (FAP) patients without a detectable germline APC mutation. V. Pethe¹, M. Croitoru¹, T. Berk^{2,3}, T. Chappell¹, J. Green⁴, Z. Cohen^{2,3}, S. Gallinger^{1,2,3}, B. Bapat^{1,2,3}. 1) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto; 2) Familial GI Cancer Registry, Mount Sinai Hospital, Toronto; 3) University of Toronto, Canada; 4) Department of Genetics, Memorial University of Newfoundland, Canada.

Germline mutations in the human base excision repair gene, *MYH*, have been shown to confer an increased susceptibility to colorectal adenomas and carcinomas among a subset of CRC patients. In particular, two non-conservative missense mutation hotspots, Y165C and G382D account for the majority of *MYH* alterations identified to date among patients affected with multiple polyposis and/or CRC. In the present study, we examined the possible contribution of *MYH* mutations in 151 Canadian multiple polyposis patients identified through Familial GI cancer registries. These patients were referred to predictive genetic testing and research program for confirmation of a possible diagnosis of FAP. Patients were previously screened by PTT assay of the entire APC coding region. None of the patients harbored a demonstrable germline APC mutation. Patients were first screened for *MYH* Y165C and G382D mutations by TaqMan- based allelotyping assays and mutations were confirmed by sequencing (Y165C) and BglIII RFLP (G382D) analysis. Thirteen of 151 (8.5%) polyposis patients harbored germline mutation at Y165C or G382D. Among these, 8 patients were biallelic Y165C mutation carriers; 4 heterozygous for G382D and one for Y165C. Heterozygotes were further screened for mutations affecting the second *MYH* allele by dHPLC analysis of the entire coding region followed by sequencing. Two heterozygotes were found to carry novel missense alterations in the 8-oxoG-recognition domain of the *MYH* gene. Interestingly, 3/8 *MYH* Y165C homozygous mutation carriers were from Newfoundland raising the possibility of this being a founder mutation in the Newfoundland multiple polyposis patient population. In summary, our data indicates that genetic screening of *MYH* should be offered to patients with a phenotype resembling FAP/AFAP in the absence of a detectable APC germline mutation.

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Cancer variation and position of BRCA1 mutation. *C.M. Phelan, P. Sun, S. Narod.* Research Womens Health, Sunnybrook & Womens Hosp, Toronto, ON, Canada.

Mutations in the BRCA1 gene have been identified in a large number of breast/ breast-ovarian cancer families. These mutations have been shown to confer a high risk of breast and ovarian cancer. However, other cancer types are also observed in these families. We have assessed the occurrence of 16 additional cancer types in 336 North American families with BRCA1 mutations scattered throughout the gene. The cancer types include colon, stomach, pancreatic, prostate, lung, kidney, bladder, thyroid, melanoma, endometrial, testicular, leukemia, lymphoma, liver, skin and cervical cancer. Our aim is to determine whether the position of BRCA1 mutation, particularly within known structural domains, influences the occurrence of additional cancer types in these families. Of the 336 families, the majority were of European descent and 126/336 families were of Ashkenazi Jewish descent. The exon 2 185delAG or exon 20 5382insC mutations were the most common, twenty mutations were located in splice sites and the remainder were deletions/insertions.

Previous studies have reported that mutations toward the 3' end of the gene are associated with lower ovarian cancer risks compared to those in the 5' portion of the gene. Our results agreed with this observation, the ovarian: breast cancer ratio in families with mutations in the 5' end (exons 1-10) = 0.69, higher than in exon 11 = 0.59 or in the 3' end (exons 13-24) = 0.37. We did not observe the highest ovarian:breast cancer ratio associated with mutations in nucleotides 2401-4190 as was reported previously. Among other cancer types, colon cancer risk appeared to be more highly associated with mutations in exon 11 compared to the remainder of the gene while lymphoma risk was highest in the 5' portion of the gene (exons 1-10).

Association of TNF and IL1 with risk of prostate cancer. *S.J. Plummer¹, X. Liu², K.L. DeJulius¹, A. Moreira¹, J.S. Witte², G. Casey¹.* 1) Dept Cancer Biology, Cleveland Clinic, OH; 2) Dept Epidemiology and Biostatistics, UC San Francisco, CA.

Both infection and chronic inflammation have been shown to influence the risk of some types of cancer. Variants in genes involved in response to infectious agents have recently been shown to associate with an increased risk of prostate cancer (RNASEL, MSR1, TLR4). We have undertaken an association study of genes involved more directly in the inflammatory process. The association of prostate cancer with variants in pro-inflammatory cytokines (TNF, IL1, IL6) and an anti-inflammatory cytokine (IL1RN) was studied in a sibling-based population of 655 cases and 482 controls. The IL1-31C variant was associated with a 2.0 fold increased risk of prostate cancer in men diagnosed over the age of 63 ($p=0.03$). The TNF-308A variant was associated with a decreased risk of prostate cancer (OR=0.6, $p=0.03$), with significantly decreased risk (OR=0.36, $p=0.006$) in men with less aggressive prostate cancers as assessed by a combination of Gleason grade and TNM stage. Neither the IL6-174 SNP or IL1RN VNTR showed association with prostate cancer in our population. Previous functional evidence indicates these promoter variants of both IL1 and TNF result in increased levels of the individual pro-inflammatory cytokine, even though their effect on prostate cancer risk is opposing. While TNF plays an important role in inflammation, it also plays diverse roles in other aspects of the innate immune system, including apoptosis and induction of the adaptive immune response. TNF has also been shown to have both tumor promoting and anti tumor effects *in vivo*. These data indicate that a variety of pathways within the innate immune system may be of importance in the etiology of prostate cancer.

***MLH1* and *MSH2* polymorphisms and colorectal cancer risk: A molecular genetic and epidemiological study.** S. Raptis, J. Knight, V. Pethe, Y.M. Chan, M. Manno, S. Gallinger, J. McLaughlin, B. Bapat. Samuel Lunenfeld Research Institute, Mount Sinai Hospital, University of Toronto, Toronto, ON.

Colorectal cancer (CRC) occurs in 1 in every 20 individuals, and is the second leading cause of cancer-related deaths of both men and women in North America. CRC may be inherited, familial, or sporadic in origin, with the sporadic form accounting for 70-80% of all CRCs. One of the most commonly inherited forms of CRC is hereditary nonpolyposis colorectal cancer (HNPCC), an autosomal dominant syndrome characterized by tumors with a genome-wide microsatellite instability (MSI) and caused by germline mutations in the mismatch repair (MMR) genes. Mutations in two MMR genes, *MLH1* and *MSH2*, account for almost 90% of cases among HNPCC patients with known MMR mutations. Previous studies have identified a number of single nucleotide polymorphisms (SNPs) in MMR genes, yet currently, little is known about the functional role of most of these SNPs, or of any relationship that may exist between their presence and the incidence of CRC. Our study addresses this issue using a case-control design to determine the population frequency of the selected SNPs in *MLH1* and *MSH2*, and to examine whether these SNPs have any underlying determinative effect in the incidence of CRC. MMR polymorphisms were selected through the review of public databases, literature annotations, and their location within the functional domains of the genes. Possible interactions among candidate SNPs and family history, MSI status, the age of onset, and the location of the CRC tumor is being examined. The analysis was performed using lymphocyte DNA isolated from over 1000 CRC cases identified through a population-based familial CRC registry, and over 1000 unaffected controls, from Ontario, Canada. Selected SNPs were genotyped using the 5' nuclease allelic discrimination, or TaqMan, assay. Preliminary observations for the selected *MLH1* SNPs- I219V, -93G>A, and the *MSH2* SNP- G322D, indicate no association with the incidence of CRC. However, a possible association with family risk is being further investigated. Results of this study will assist in further elucidating the role of MMR genes in colorectal cancer susceptibility.

Genomic rearrangements in the APC tumor-suppressor gene in familial adenomatous polyposis. *B. Roa¹, E. Chin¹, B. Chong¹, M.A. Young¹, P. Ward¹, C.S. Richards², M. Hegde¹.* 1) Medical Genetics Laboratories, Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) OHSU DNA Diagnostic Lab Oregon Health & Science University 3181 SW Sam Jackson Park Road, MP-350.

Germline mutations of the adenomatous polyposis coli (APC) tumor-suppressor gene result in familial adenomatous polyposis (FAP). The majority of identified APC mutations are single-nucleotide changes, or insertions or deletions of a few bases that result in premature protein truncation. Intragenic APC gene rearrangements have been reported but not well characterized. We performed dosage analysis for APC gene rearrangements in 26 patients with a clinical diagnosis of FAP, who had tested negative by DHPLC mutation scanning and/or bi-directional sequence analysis of the entire APC coding region. Dosage studies were performed initially by Multiplex Ligation-dependent Probe Amplification (MLPA) and confirmed using real-time quantitative PCR analysis of individual exons. APC deletions were detected in ten patients; four patients appear to have a heterozygous deletion of the entire coding region, while six patients showed partial deletions which include one or more exons of the APC gene. In one intriguing case, dosage studies provided evidence of a novel heterozygous duplication that involves exon 4 of APC. Exon 4 appears to be an endpoint for two deletions and one duplication mutation identified in our study, which could suggest a recombination hotspot in the region. The identification of ten genomic rearrangements in this study suggests that deletions and duplications can account for as much as 24% of germline mutations in APC. A diagnostic strategy that includes mutation scanning or sequencing in combination with testing for rearrangements in the APC gene could enhance the test sensitivity as much as 97%. It is interesting that all four patients with heterozygous deletions of the entire coding region are indicated to have an attenuated FAP phenotype. Additional studies are necessary to define the exact frequency of APC gene rearrangements, and to elucidate mutation genotype-phenotype correlations for Familial Adenomatous Polyposis.

ONCOGEN c-erbB-2 AMPLIFICATION IN MALIGNANT BREAST TUMOR IN RELATION WITH HISTOLOGIC PARAMETERS AND SURVIVAL FREE FOR ILLNESS. *A. Rojas-Atencio¹, I. Valbuena¹, K. Urdaneta¹, L. Gonzalez², M. Soto-Quintana¹, F. Alvarez-Nava¹.* 1) Genetica, Univ del Zulia, Zulia, Zulia, Venezuela; 2) Oncology Service Hospital Clinico de Maracaibo.

Recent advances in the understanding of the underlying molecular and genetic alterations in the development and progression of breast cancer has provided the opportunity to develop novel therapeutic strategies for this disease. None of these developments has had a greater recent impact on the c-erb B-2 oncogene. The objective of this study was to evaluate the amplification of the c-erb B-2 oncogene by FISH, relating IHC positive patients with FISH analysis according to histological gradation, presence of positive lymph nodes and evolution of the illness. We analyzed 110 patients by FISH who were positive to amplification c-erb B-2 oncogene by IHC. Our finding indicated that positive amplification c-erb B-2 oncogene had a worse prognosis, than those patients who presented only amplification c-erb B-2 by IHC. And another hand we found a bigger number of positive cases by FISH with histological gradation II and III, as well as in those patients who had infiltration in more than 6 lymph nodes and disease evolution. We conclude that is necessary to emphasize the importance that FISH has in the identification of c-erb B-2 oncogene, mainly if we take into account that an election of therapeutic strategies exists to patients that are inside this group of poor prognosis.

Familial and sporadic Non-Medullary Thyroid Carcinoma. *G. Romeo*¹, *E. Bonora*¹, *C. Evangelisti*¹, *K. Stankov*¹, *G. Lenaz*². 1) Lab. Genetica Medica; 2) Dept. of Biochemistry University of Bologna Medical School.

Thyroid cancers are relatively rare, with age-adjusted incidence rates ranging from 0.9-5.2/100,000 cases per year. At least 80% of these are papillary thyroid carcinomas, more common in women. The major known risk factor for sporadic papillary forms is prior exposure to radiation, with dose-dependent effects. RET rearrangements were identified as specific events in sporadic papillary thyroid carcinogenesis. Familial non-medullary thyroid carcinoma (FNMTC) is characterized by more aggressive phenotype than its sporadic counterpart, and represents 3%-7% of all thyroid carcinomas. Transmission of susceptibility is compatible with autosomal dominant inheritance with reduced penetrance, or with complex inheritance. Thanks to the collaboration of the International Consortium for the Genetics of FNMTC we collected so far 220 pedigrees with two or more affected individuals. Using this unique resource we mapped two predisposing loci, NMTC1 locus (Non Medullary Thyroid Carcinoma) on 2q21 and TCO locus (Thyroid tumors with Cell Oxyphilia) on 19p13.2. The latter locus is associated with recurrence of oxyphilic tumors, characterized by the presence of Hürthle cells, rich in mitochondria. Genes mapping to 19p13.2 were studied to identify TCO susceptibility variants: EDG5 (sphingosine receptor), CYTC (cyt c heme binding), LASS1 (homolog of longevity assurance factor), NIS (sodium iodide symporter). New missense changes were identified in EDG5 and CYTC, but they did not cosegregate with the tumor phenotype and were found with similar frequency in controls. Further analysis of candidate genes is in progress. In parallel, functional studies on XTC-1 cells, derived from oxyphilic thyroid tumors, showed differential activity in mitochondrial respiratory complexes and increased production of reactive oxygen species (ROS), compared to B-CPAP cells derived from papillary thyroid tumor. Recently, a somatic mutation in BRAF gene, coding for a kinase acting in the RET/MAPK pathway, was identified in sporadic papillary thyroid carcinoma. We are currently screening a panel of 250 diverse sporadic thyroid tumors to identify BRAF mutations in our sample.

MEN1-related pancreatic insulinomas can develop in the absence of chromosome instability or microsatellite instability. *P.C. Scacheri¹, A.L. Kennedy¹, K. Chin², M.T. Miller¹, J.G. Hodgson², J.W. Gray², S.J. Marx³, A.M. Spiegel⁴, F.S. Collins¹.* 1) National Human Genome Research Institute, NIH, Bethesda, MD; 2) UCSF Comprehensive Cancer Center, San Francisco, CA; 3) NIDDK, NIH, Bethesda, MD; 4) NIDCD, NIH, Bethesda, MD.

Multiple endocrine neoplasia, type I (MEN1) is an inherited cancer syndrome characterized by tumors arising primarily in endocrine tissues. The responsible gene acts as a tumor suppressor, and tumors in affected heterozygous individuals occur following inactivation of the wild-type allele. Previous studies have shown that Men1 knockout mice develop multiple pancreatic insulinomas, but this occurs many months after loss of both copies of the Men1 gene. These studies imply that loss of Men1 is not alone sufficient for tumor formation, and that additional somatic genetic changes are most likely essential for tumorigenesis. To gain insights to these additional mutations and the mechanisms through which they may be generated, we used array-based comparative genomic hybridization (array CGH) to analyze genomic copy number aberrations in pancreatic insulinomas from Men1 knockout mice. We found that homozygosity for the Men1 allele in tumors often occurs by duplication of the mutant Men1 allele. Although a few tumors showed evidence of chromosomal instability, the majority of Men1-null tumors were apparently chromosomally stable. Evidence for microsatellite instability was also not found in Men1-null tumors. Thus, the somatic genetic changes that are postulated to lead to tumorigenesis in a mouse model of MEN1 are likely to occur either at the nucleotide level, or through epigenetic mechanisms that lead to gene silencing or activation. The lack of detectable CIN and MIN in these Men1-insulinomas is in stark contrast to the situation for many solid tumors.

The Hereditary Paraganglioma syndromes: A small series of patients with SDHB and SDHD mutations. K.

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About 20% of paragangliomas (hnpgl) and pheochromocytomas (pheo) are caused by dominantly inherited gene mutations. Hnppls can present as non-secreting head and neck neoplasms and pheos can present as catecholamine-secreting adrenal or extra-adrenal tumors. Hnppls and pheos are recognized features of several cancer syndromes including: VHL, MEN2, NF1, and the newly recognized Hereditary Paraganglioma syndromes, PGL1 and PGL2. The PGL1 and PGL4 syndromes are caused by SDHD and SDHB mutations respectively. We describe a series of 10 pheo and hnppl patients with detectable SDHD and SDHB mutations. Mean age of diagnosis among our patients was 33 years (range: 19-45) and presenting symptoms included hypertension, panic attacks, palpitations, headaches, fatigue and swollen lymph nodes. Two patients had SDHD mutations and eight patients had SDHB mutations. Both SDHD mutation carriers had hnppls (in one case, also pheo) and all SDHB-mutation carriers had extra-adrenal pheos. One patient had metastatic disease at presentation and four patients had multiple tumors at presentation or subsequent exams. Four patients reported relatives with pheos or hnppls, although some of these cases were identified after the patients initial diagnoses. Among SDHB mutation carriers, three other cancers were reported among relatives: gastric tumor, renal cell carcinoma (RCC), and papillary thyroid carcinoma. RCC is a recognized feature of PGL4; significance of the other tumors is unclear. Annual monitoring for pheos and hnppls was recommended to at-risk relatives, beginning in adolescence, and predictive genetic testing was also offered. PHEO and HNPGl patients should be referred for genetic counseling, because the identification of inherited pheos and hnppls has important monitoring implications for at-risk relatives.

Second primary nervous system tumours after optic pathway gliomas in Neurofibromatosis Type 1: increased risk after radiotherapy. *S. Sharif*¹, *R. Ferner*², *M. Upadhyaya*³, *J. Birch*⁴, *G. Evans*¹. 1) Genetics, Manchester; 2) Neurology, London; 3) Genetics, Cardiff; 4) CTR, Manchester.

Optic pathway gliomas (OPG), the commonest CNS tumour in NF1 mainly occur in childhood. The implications of OPGs and related treatments in long-term survivors are becoming apparent. The risk of developing further CNS tumours (CNST) is noted. We reviewed all NF1 OPG patients from 2 NF1 clinics to evaluate the risk of subsequent CNSTs and implications of radiotherapy therapy. In one centre (Manchester), we compared the incidence of gliomas in NF1 OPG patients to matched NF1 controls without OPGs, whose follow up was censored when the OPG patients developed their second gliomas. These patients were checked on the cancer registry, to ensure cancer ascertainment. 80 NF1 OPG patients were identified. All case notes were reviewed. 29 were seen, serial neuroimaging reviewed and mutation analysis performed. Prevalence of OPGs was 5.8%, 39% received treatment (72% had radiotherapy alone or in combination). 11/25 had pathogenic mutations clustering towards the 5' and center of the NF1 gene. Second tumour analyses of 58 NF1 OPGs patients, (limited data on 16; OPG excluded in 3; 3 gliomas developed prior to OPG, also excluded) found 26% developed a subsequent non-OPG glioma (average 10.9 years post OPG). Significantly fewer gliomas, 1, occurred in follow up of 162 matched controls without OPGs ($P < 0.001$). 47% of NF1 OPGs with second tumours received radiotherapy, average 14.4 years post radiotherapy. 5 malignant peripheral nerve sheath tumours (MPNST) were seen in 4 NF1 OPG patients, 3 had radiotherapy. 9 of the 18 patients who had radiotherapy after the OPG developed 12 second tumours (gliomas and MPNSTs) in 307.9 years of follow up after radiotherapy. In those without radiotherapy ($n=40$), 8 patients developed 9 tumours in 721.1 years of follow up after diagnosis of the OPG ($p < 0.01$). There is a significantly increased risk of gliomas occurring after OPG. Many were treated with radiotherapy, which may thus be a possible factor. We previously reported the increased risk of NF1 MPNSTs post radiotherapy. This data suggests that radiotherapy should only be used if absolutely essential in NF1.

A promoter SNP in the MLH1 gene is associated with mismatch repair deficient colorectal cancer. *J.A. Shorto¹, J.M. Allan², R. Coggins³, D. Fletcher¹, R. George¹, M. Katory¹, M. Meuth¹, G. Smith⁴, D.T. Bishop³, A. Cox¹.* 1) Institute of Cancer Studies, University of Sheffield Medical School, UK; 2) Epidemiology and Genetics Unit, Department of Biology, University of York, UK; 3) Genetic Epidemiology Division, Cancer Research UK Clinical Centre, Leeds, UK; 4) Biomedical Research Centre, Ninewells Hospital and Medical School, Dundee, UK.

One pathway to colorectal cancer (CRC) malignancy involves the mutation or loss of expression of a DNA mismatch repair (MMR) gene causing microsatellite instability (MSI) and a mutator phenotype. This leads to further mutations and eventually cancer. This pathway is followed in cases of HNPCC and a subset of sporadic CRC patients. In the majority of MMR deficient sporadic cases the mechanism of MMR inactivation is via methylation of the MLH1 gene promoter. A G>A polymorphism at position -93 is located in the core promoter region of MLH1, adjacent to several CpG sites that, when methylated, have been shown to associate with loss of MLH1 expression and CRC development. We hypothesized, therefore, that the -93 variant may also be involved in the pathogenesis of MMR deficient CRC. We genotyped 688 histopathologically confirmed CRC cases and 592 population based controls using the 5' nuclease PCR assay for the G>A SNP at position -93 in the MLH1 gene promoter. The cases were categorized into MMR deficient and MMR proficient tumours using MSI analysis of normal and tumour DNA, and immunohistochemical analysis of MLH1 and MSH2 proteins in tumour sections. There was no association between the -93 SNP and CRC overall OR (odds ratio) = 1.05 (95 % confidence interval (CI) 0.8, 1.4). However we found that the presence of an A allele at -93 is associated with MMR deficient CRC tumour development, OR = 1.8 (95 % CI 1.1, 3.0). Furthermore, a dose response is seen, with ORs of 1.5 (95 % CI 0.8, 2.7) and 5.1 (95 % CI 1.8, 14.2) when one and two A alleles respectively are present. These data suggest that a polymorphism in the promoter region of MLH1 may influence MMR deficient tumour development. Further work is in progress to confirm this result and determine whether the G to A change at -93 affects the methylation status of the MLH1 promoter.

Evaluation of Glutathione-S-Transferase Polymorphisms as Biomarkers of Cancer Risk Among Filipinos: A Preliminary Study. *C.T. Silao¹, E.C. Cutiongco¹, C.D. Padilla¹, C. Ngelangel²*, *Philippine Cancer Genetics Research Group*. 1) Institute of Human Genetics, National Institutes of Health Philippines, University of the Philippines Manila; 2) Oncology Study Group, Institute of Clinical Epidemiology, National Institutes of Health Philippines, University of the Philippines Manila and Section of Medical Oncology, Department of Medicine, Philippine General Hospital and Jose R. Reyes Memorial Medical Center.

This is a molecular epidemiologic study that looks at the putative association between specific genetic polymorphisms and cancer susceptibility. Genetic polymorphism studies were done in the following metabolizing enzymes: GSTM1, GSTP1, and GSTT1. Corresponding demographics, medical histories, social histories, diet histories, and occupational exposure data were collected. Cancer sites included four (4) of the top ten cancers among Filipinos - breast, lung, colorectal, and oral cavity. This initial report focuses on polymorphic genes encoding for glutathione-S-transferases (GST) which have been reported to be involved in modifying cancer risk in human populations. Specifically, we investigated the following GST isoforms: GSTM1, GSTT1, and GSTP1. Frequencies of GSTM1 and GSTT1 null genotype were identified using polymerase chain reaction (PCR), while that of the GSTP1 exon 5 polymorphism was determined using PCR and restriction fragment length polymorphism (RFLP) of DNA obtained from blood samples of Filipino cancer patients and control subjects. Initial frequencies of GSTM1 null genotype (n=891) were more prevalent for both the case (60%) and control (64%) groups than the wild type. GSTT1 polymorphic deletion (n=151) was present in 41-60% of the case groups as compared to the control groups (25-43%). The frequencies of homozygous mutants GSTP1 gene (Ile105Val) did not differ between the case groups (4-9%) and the control groups (5-8%). Among the three GST isoforms, the gene for GSTT1 shows the most potential as a cancer biomarker based on genotypic frequencies of its polymorphic deletion as compared to GSTM1 and GSTP1. More subjects and studies are needed to determine the significance of these initial findings.

Analysis of polymorphisms in DNA repair genes as modifiers of breast cancer risk in BRCA1 and BRCA2 carriers. P. Smith¹, A. Spurdle², P.A. Harrington¹, F. Durocher³, D. Hughes⁴, S. Ginolhac⁴, O. Sinilnikova^{4,5}, C. Szabo⁴, J. Labrie³, I. Coupier⁶, D. Stoppa-Lyonnet⁶, S. Peock¹, M. Cook¹, J.L. Hopper⁷, J. Simard³, D.E. Goldgar⁴, A.M. Dunning¹, G. Chenevix-Trench², D.F. Easton¹, ABCFS, AJBCS, EMBRACE, kConFab, GGC-France and INHERIT collaborators. 1) CR-UK Genetic Epidemiology Unit and Human Cancer Genetics Research Group, University of Cambridge, Strangeways Research Laboratory, Cambridge, U.K; 2) Queensland Institute for Medical Research, Brisbane, Australia; 3) CHUL Research Centre, Laval University, Quebec City, Canada; 4) International Agency for Research on Cancer, Lyon, France; 5) Plate-forme mixte de génétique constitutionnelle des cancers fréquents, Hospices Civils de Lyon - Centre Lon Bérard, Lyon, France; 6) Service de Génétique Oncologique, Institut Curie, Section Mdicale, Paris, France; 7) Centre for Genetic Epidemiology, University of Melbourne, Melbourne, Australia.

Estimates of breast cancer risk in BRCA1 and BRCA2 mutation carriers vary according to the degree of family history, suggestive of modification of cancer risk by other genes. Previous studies have suggested that polymorphisms in RAD51, whose protein product interacts with both BRCA1 and BRCA2, are associated with an increased risk of breast cancer in BRCA2, but not BRCA1, carriers. We genotyped two polymorphisms RAD51 135g>c and 172g>c, together with polymorphisms in DNA repair genes XRCC1, XRCC2, XRCC3, XPD and LIG4, in 1112 BRCA1 and 485 BRCA2 carriers (905 affected with breast cancer, 692 unaffected) from Australia, Canada, USA and Europe. Associations were assessed using Cox Regression and a modified segregation analysis. In contrast to previous analyses, we found no evidence of an increased breast cancer risk in RAD51 135c carriers, in either BRCA2 carriers (hazard ratio 1.31, 95%CI 0.86-1.99) or BRCA1 carriers (HR 0.97, 95%CI 0.79-1.20) . We found some evidence of a higher breast cancer risk (based on 10 carriers) in homozygotes for RAD51 135c (HR 2.81, 95%CI 1.71-4.64). We found no evidence of an association with any of the other 10 SNPs tested. We conclude that these polymorphisms in DNA repair genes are not strongly associated with breast cancer risk in BRCA1 or BRCA2 carriers.

A multi-centre study of cancer incidence in CHEK2 1100delC mutation carriers. *D. Thompson¹, N. Rahman³, M. Stratton³, M. Schutte⁴, H. Meijers-Heijboer⁴, R. Houlston³, R. Eeles³, J. Chang-Claude⁵, A. Dunning², D. Easton¹, CHEK2-Breast Cancer Consortium.* 1) CR-UK Genetic Epidemiology &; 2) CR-UK Human Cancer Genetics Group, Univ. Cambridge, UK; 3) Cancer Genetics Section, Institute of Cancer Research, UK; 4) Dept. Clinical Genetics & Dept. Medical Oncology, Erasmus MC, Rotterdam; 5) Div. Clinical Epidemiology, Deutsches Krebsforschungszentrum, Heidelberg.

The CHEK2 1100delC mutation is associated with an approximately 2-fold increase in breast cancer risk. It has recently also been suggested to be associated with increased risks of colorectal and prostate cancer, but its involvement in these or other types of cancer has not yet been definitely established. We collected cancer information for 706 non-BRCA1/2 breast cancer families (10,726 individuals) from the UK, Germany, Netherlands and the USA. At least one person per family was tested for CHEK2 1100delC, and carriers were found in 38 families. Relative risks (RR) to mutation carriers and non-carriers in the cohort were estimated by maximum likelihood, via the EM-algorithm to allow for unknown genotypes. Excluding breast and ovarian cancer, the cancer risk was borderline significantly higher in carriers than non-carriers (carrier:non-carrier RR ratio=1.8, 95% CI=1.0-3.3). The ratio was 1.1 in females (CI=0.6-2.0), but there was some evidence of a higher risk in male carriers (ratio=2.6, CI=0.9-7.2). The excess carrier risk was more evident before age 60 (ratio=2.2, CI=0.8-6.2) than at older ages (ratio=1.4, CI=0.7-2.7). There was no evidence of an increased risk of ovarian cancer (carrier RR=0.0) or prostate cancer (carrier RR=1.7, CI=0.2-12.5), two sites known to be associated with BRCA1/2 mutations. Colorectal cancer was the only specific site with a significantly higher risk in carriers than in the general population (RR=2.7, CI=1.2-6.1). Additional analyses based on 429 colon cancer cases, 1287 prostate cancer cases and 3696 controls found no associations (colon odds ratio (OR)=0.3, CI=0.0-2.3; prostate OR=0.8, CI=0.4-1.8). We conclude that risks of other cancers in CHEK2 1100delC carriers, if any, are likely to be smaller than for breast cancer.

***MSH6* in breast cancer families with phenotypic features of HNPCC.** P. Vahteristo¹, S. Ojala¹, A. Tamminen¹, J. Tommiska¹, H. Sammalkorpi², S. Kiuru-Kuhlefelt^{2, 4}, H. Eerola³, L.A. Aaltonen², K. Aittomaki^{2, 4}, H. Nevanlinna¹. 1) Dept. Obstetrics & Gynecology, Helsinki University Central Hospital, Helsinki, Finland; 2) Dept. Medical Genetics, Helsinki University, Helsinki, Finland; 3) Dept. Oncology, Helsinki University Central Hospital, Helsinki, Finland; 4) Dept. Medical Genetics, Helsinki University Central Hospital, Helsinki, Finland.

The role of *MSH6* gene for breast cancer predisposition is unknown. Studies on breast cancer risk in HNPCC have given conflicting results and have not been focussed on *MSH6*-carrier families, while breast cancer cases have been described in such families. *MSH6* protein is part of the so called BASC complex (BRCA1-associated genome surveillance complex), which is comprised of BRCA1-associated DNA repair proteins and suggested to serve as a sensor for DNA damage. This functional association with BRCA1 may suggest a role for *MSH6* also in breast cancer predisposition. We and others have also described patients with both breast and colorectal carcinoma and germline *MSH6* mutations. Here we have analyzed a series of 38 breast cancer families with phenotypic features of HNPCC for germline mutations in the *MSH6* gene. No disease associated mutations were detected among breast cancer families. However, a previously reported putatively breast cancer associated Finnish *MSH6* mutation was found in an atypical HNPCC-family, and identified as a novel Finnish *MSH6* founder mutation. The occurrence of the mutation was further evaluated in an extensive set of familial breast and familial colorectal cancer cases. No additional mutation carriers were found. In conclusion, the absence of germline *MSH6* mutations in our set of breast cancer families suggests that *MSH6* mutations do not underlie, or may be very rare, in breast cancer families with phenotypic features of HNPCC. In addition, the new founder mutation Glu995STOP may have a very limited, regional contribution to colorectal cancer in Finland.

Expression of Zic 1 in desmoid tumors. *K. Van Dam*¹, *A. Bastidas*¹, *S. Amini Nik*¹, *A. Jadidizadeh*¹, *R. Scior*², *S. Tejpar*¹, *J-J. Cassiman*¹. 1) Center for Human Genetics, University of Leuven, Leuven, Belgium; 2) Department of Morphology and Molecular Pathology, University of Leuven, Leuven, Belgium.

Zic genes are the vertebrate homologs of *Odd-paired*, the *Drosophila* pair-rule gene. Recent studies of different vertebrate Zic homologs have provided evidence that Zic genes play multiple roles during neural development. They control the differentiation of ectoderm into neuroectoderm, the process of neurulation and the morphogenesis of the CNS. Next to the important and conserved role for Zic genes in neural development, additional expression patterns have been reported in the somitic mesoderm during development of vertebrates. Additionally, Zic-related genes in lower chordates also appear to have additional functions in mesenchyme development. Because of these findings, it was postulated that the Zic family of genes has evolved towards 2 different functions: a well conserved function during the development of neural tissues and an additional function in mesenchymal tissues, although the role for Zic genes in the latter tissues is not yet clear.

In humans, expression of Zic 1 was detected in the developing and adult cerebellum and in medulloblastomas. Because of this specific expression pattern, Zic 1 was suggested to be a potential biomarker of medulloblastomas. In the present study, we report the expression of Zic 1 in desmoid tumors. Desmoid tumors are benign, soft-tissue tumors composed of fibroblast-like cells. We confirmed the high expression levels of Zic 1 in desmoids at the mRNA and protein level and showed the absence of Zic 1 expression in their putative progenitor cells (the fascia). Additionally, Zic 1 expression was found in other fibro-proliferative processes and a possible relationship between Zic 1 expression levels and the proliferation status of fibroblasts was found. In conclusion, we show that the expression of Zic 1 in human tumors is not limited to medulloblastomas and we suggest a possible role for Zic 1 in the proliferation of mesenchymal cells.

Increased risk of breast cancer due to a hyper mutable tract in BRCA2 caused by N863K? *G.R. Vink, A.H.J.T. Bröcker-Vriends, C.J. van Asperen, M.H. Breuning, E. Bakker.* Center for Human and Clinical Genetics, LUMC, Leiden, Netherlands.

Truncating APC mutations are responsible for FAP and mutations in the BRCA1 and BRCA2 genes are responsible for hereditary breast and ovarian cancer. Missense mutations form a great problem in the genetic counseling because of their often unknown pathogenicity. With the PT-test normally only truncating mutations can be detected. However, about 6% of the Ashkenazi Jews are carrier of a missense mutation in the APC gene, which causes a truncated APC protein in the PT-test (c.3920T>A, p.I1307K). Most likely, this truncation is an in vitro phenomenon caused by the T>A transversion. The (A)8 tract, which is formed by this transversion, is not only unstable in vitro, but also in vivo, leading to somatic mutations. Rather than altering the function of the encoded protein, this variant creates a small hyper mutable region in the gene, indirectly causing an increased cancer predisposition. It is thought that I1307K increases the risk for colorectal cancer about 1.5 to 2 times. We identified a similar, but much more uncommon variant in the BRCA2 gene: c.2817T>A, p.N863K. This missense mutation causes, like I1307K in the APC-gene, a truncated BRCA2 protein in the PT-test and also creates an extended adenine nucleotide tract (A8). We therefore believe that the N863K variant in BRCA2 is responsible for the impairment of replication fidelity and increases the cancer risk of carriers by forming a mutational hotspot.

Assessment of the clinical significance of IVS2-6 T >A in BRCA1. *M.P.G. Vreeswijk¹, L. van Driel¹, J.N. Kraan¹, G.R. Vink¹, C.J. van Asperen¹, J. Morreau², C.J. Cornelisse², P. Devilee^{1,2}.* 1) Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, the Netherlands; 2) Department of Pathology, Leiden University Medical Center, Leiden, the Netherlands.

Germ-line mutations in the breast cancer susceptibility gene BRCA1 account for a large proportion of hereditary breast/ovarian cancer families. A large number of disease-causing germ-line mutations and variants of unknown pathological significance have been identified. Missense and intron variants cannot be readily distinguished as either disease-associated mutations or benign polymorphisms, and are classified as variants of uncertain clinical significance (VUCS). Characterization of the clinical significance of such variants is important for the medical management of patients carrying these variants, as well as for their family members.

Recently, the IVS2-6 T>A intronic change was detected in the BRCA1 gene of a young woman diagnosed with breast cancer at the age of 30. Histological examination of resected breast tissue showed a grade 3 ductal carcinoma with lymphocytic infiltrate and prominent pushing margins, which have been described as hall marks for BRCA1 related tumours. Her mother died of ovarian cancer age 55, whereas her mothers sister died of breast cancer at the age of 34. Splice-site prediction programs predicted a shift of the acceptor splice site of exon 3 with four basepairs upstream of the original start site. Sequence analysis of cDNA from fibroblasts derived from a carrier of the variant, confirmed the altered splice site, leading to an out-of-frame fusion of BRCA1 exon 2 and 3, introducing a stopcodon at the start of exon 3. Preliminary data concerning DNA analysis of the tumour material show LOH of the wildtype allele. Although the analysis of cosegregation is still in progress, these data already strongly suggest that the IVS2-6 T > A intronic change is a pathogenic mutation, predisposing carriers to breast and ovarian cancer.

The analysis of other variants of uncertain clinical significance in BRCA1 is in progress.

Localization of folliculin, the BHD gene product, by cell fractionation studies. *M.B. Warren¹, M. Baba¹, D. Esposito², M.L. Nickerson¹, N. Sharma¹, W. Zhen¹, L.S. Schmidt², B. Zbar¹.* 1) Laboratory of Immunobiology, NCI-Frederick, Frederick, MD, 21702; 2) PEL and BRP, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD, 21702.

The Birt-Hogg-Dubé syndrome (BHD) is a rare inherited genodermatosis characterized by benign tumors of the hair follicle (fibrofolliculomas), pulmonary cysts, spontaneous pneumothorax, and an increased risk for renal carcinoma. The majority of BHD-associated renal tumors are oncocytic hybrids, with features of chromophobe renal carcinoma and oncocytoma. The BHD gene encodes a novel protein, folliculin, with no homology to any known human proteins or functional domains, but is conserved across species. We investigated the subcellular localization of endogenous folliculin in HEK-293 cells and also the location of HA-tagged folliculin in stably-transfected, tetracycline-inducible HEK-293 cells. We obtained cell fractions by differential centrifugation and evaluated the fractions for folliculin by Western blotting with a polyclonal folliculin-directed antibody. The integrity of cell fractions was monitored with antibodies to cytoplasmic, nuclear, mitochondrial and microsomal proteins. The location of endogenous folliculin in HEK-293 cells and HA-folliculin, in stably-transfected HEK-293 cells induced with tetracycline, was predominantly in the cytoplasm. Future experiments with cell fractions from synchronized cells will evaluate the expression level and localization of folliculin during the cell cycle. Funded in part by DHHS# NO1-CO-12400.

Prevalence of *BRCA* Mutations and Founder Effect in High-Risk Hispanic Families in Southern California. J. Weitzel¹, K. Blazer¹, R. Nelson¹, V. Lagos¹, C. Ricker¹, J. Herzog¹, S. Neuhausen². 1) City of Hope Cancer Center, Duarte, CA; 2) University of California, Irvine, Irvine, CA.

Approximately 12% of the US population is Hispanic with the majority residing in urban centers such as Los Angeles. Breast cancer (BC) is the leading cause of cancer death in female Hispanics. The prevalence of *BRCA* mutations among high-risk Hispanic families is unknown. **Methods:** Eighty-five individuals of Hispanic origin, with a personal or family history of breast and/or ovarian cancer, were enrolled in an IRB-approved registry and underwent cancer risk assessment including *BRCA* testing. Mutation probability was calculated. Haplotype analyses were performed if mutations were observed in two or more unrelated families. **Results:** Seventy-two (85%) probands had BC, 3 (4%) had DCIS, 4 (5%) had ovarian cancer, 1 (1%) had peritoneal cancer and 5 (6%) had a family history but were unaffected with cancer. Mean age at BC diagnosis was 37 (range=23-59). Mean pre-test mutation probability of all 3 models combined was 21% (range=4-70%). Overall, 27 (32%) had deleterious mutations (20 in *BRCA1*, 7 in *BRCA2*), 23 (27%) had one or more unclassified variants, and 35 (41%) had negative results. The average mutation probability of the *BRCA* carriers compared to non- or variant carriers was 33% (sd=21%) and 15% (sd=12%) respectively (p<0.001). The most common deleterious mutation was 185delAG (4 of 27, 15%). The Hispanic 185delAG carrier families share the same haplotype from D17s1320 through *BRCA1*, as do three reference Ashkenazi Jewish families. Haplotype analyses of 3 other recurrent *BRCA1* mutations, IVS5+1GA (n=2), S955X (n=2) and R1443X (n=3), also suggest founder effects. R1443X was previously reported in a Spanish family. **Conclusion:** This is the largest study of Hispanic families in the US, and the results suggest that they have similar model-based *BRCA* mutation probabilities as other studied populations. The *BRCA1*185delAG mutation was prevalent (4.7%) in this clinic-based cohort of predominantly Mexican descent, and shared the Ashkenazi Jewish founder haplotype. We speculate that these individuals may be descendants of Spanish Jews who converted to Christianity to avoid persecution.

Familial renal carcinoma: clinical subtypes and risk for renal carcinoma development. *B. Zbar*¹, *G. Glenn*², *P. Choyke*², *M. Walther*², *J. Peterson*², *W.M. Linehan*². 1) Laboratory of Immunobiology, NCI- Frederick, Frederick, MD; 2) Urologic Oncology Branch, NCI, Bethesda, MD.

Familial renal carcinoma (FRC) refers to families with two or more family members affected with renal cell carcinoma who are not affected with the known forms of inherited renal carcinoma. We reviewed the clinical records of all renal carcinoma families evaluated at the Clinical Center, National Institutes of Health over the past 14 years to define clinical features of affected families and to determine the risk of renal carcinoma development in unaffected first-degree relatives. FRC families could be subdivided by clinical characteristics into several distinct subgroups based on renal tumor histology and tumor multiplicity. The histologic types and distribution of renal tumors found in the FRC subgroups was as follows: 1) single clear cell renal carcinomas; 2) bilateral, multiple clear cell renal carcinomas; 3) single clear cell renal carcinomas and renal oncocytomas; 4) single clear cell renal carcinomas and papillary renal carcinomas; 5) single and multiple renal oncocytomas without the other clinical features of the Birt-Hogg-Dube' syndrome; 6) single or multiple bilateral papillary renal carcinomas; and 7) other. We evaluated 101 first degree unaffected, at-risk members of 24 FRC families for occult renal tumors by computerized tomography and renal ultrasound. We detected 3/101 at-risk members of FRC families to have an occult renal neoplasm. A clear cell renal carcinoma was detected in one patient; in a second patient, a renal oncocytoma was detected. The renal tumor in the third patient was 1 cm in diameter and has not, as yet been resected.

Novel Mutations in FH associated with hereditary leiomyomatosis and renal cell cancer in families in North

America. *M.H. Wei^{1,2}, O. Toure², O. Jian², G. Glenn², C. Torres-Cabala³, L. Middelton⁴, M. Merino³, M. Walther⁴, C. Stolle⁵, L. Schmidt⁶, B. Zbar⁷, M. Linehan⁴, J. Toro².* 1) Program of Division of Cancer Epidemiology and Genetics, SAIC-Frederick, Inc., National Cancer Institute Frederick, Frederick MD; 2) Genetics Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD 20892; 3) Laboratory of Pathology, National Cancer Institute, Bethesda, MD20894; 4) Urologic Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20894; 5) Children's Hospital of Philadelphia, Philadelphia, PA; 6) Basic Research Program, SAIC-Frederick, Inc., National Cancer Institute Frederick, Frederick, MD 21702; 7) Laboratory of Immunobiology, National Cancer Institute, Frederick, Frederick, MD 21702.

Hereditary leiomyomatosis and renal cell cancer (HLRCC) (OMIM 605839) is the autosomal dominant predisposition to the development of smooth muscle tumors of the skin and uterus and/or renal cancer. We previously showed that mutations in the fumarate hydratase gene (FH) are associated with HLRCC in families in North America. In this study we characterize the clinical and genetic features of 17 new families with HLRCC in North America. Sequence analysis revealed 11 germline mutations in 17 new families with HLRCC. Of these 11 mutations, 6 were novel. These 6 novel mutations consisted of: four missense (A349G, C568T, C1025A, T1126C), one insertion (A ins@ 111) and one splice site mutation (G138+1C). Seven families with HLRCC had renal tumors. The FH mutations associated with 7 families with renal tumors consisted of: R58X in 3 families, C568T in one family, C1025A in one family, T1126C in one family and a G138+1C in one family. FH mutations were associated with a spectrum of renal tumors ranging from papillary type II renal cell carcinoma to collecting duct carcinoma of the kidney. In combination with our previous report, we have identified 28 different germline FH mutations of which 24 are novel. To date our FH mutation detection rate is 90% (47/52) in families with cutaneous leiomyomas and/or papillary or tubopapillary renal tumors with distinct cytological features.

Increased Level of Chromosomal Instability in *BRCA1* Mutation Carriers Following Irradiation Induced DNA Damage. Z. Kote-Jarai¹, A.Y. Salmon^{*1,2}, T. Menghistu¹, M. Copeland¹, K. Bishop³, A. Ardern-Jones³, S. Shanley³, The Carrier Clinic Collaborators³, Y. Lu⁴, J. Shipley⁴, R.A. Eeles^{1,3}. 1) Translational Cancer Genetics, Institute of Cancer Research, Sutton, Surrey, UK; 2) Hadassah Medical Centre, Jerusalem, Israel; 3) Royal Marsden Foundation NHS Trust, London, UK; 4) Cell Biology and Experimental Pathology, Institute of Cancer Research, Sutton, Surrey, UK.

Deleterious mutations in the *BRCA1* gene predispose women to an increased risk of breast cancer. Many functional studies have suggested that *BRCA1* has a role in DNA damage repair and failure in the DNA damage response pathway often leads to the accumulation of chromosomal aberrations. Our aim was to investigate if lymphocytes from *BRCA1* mutation carriers show an increased level of chromosomal instability (CIN) after irradiation induced DNA damage compared with non-carrier controls. Short term cultures of lymphocytes were established from fresh blood samples. Cells were irradiated using a Cobalt source at high dose rate and metaphase spreads were analysed by 24-colour chromosome painting, M-FISH. Image capturing was done by Smart Capture 2 software and chromosomal rearrangements and breaks were analysed by Quips SpectraVysion. In a pilot study we tested two different radiation doses, 4 Gy and 8 Gy, and two different time-points for metaphase spreads after the radiation treatment. The 48h time-point was selected to identify both unstable and stable chromosomal aberrations. The 6 day time-point was chosen, as only stable chromosomal alterations will be detected at this stage. We analysed 181 metaphases from 5 *BRCA1* mutation carriers and 104 metaphases from 5 non-carrier control samples 6 days after 8Gy of irradiation. We found that 7.52 % (0.51) of the chromosomes were involved in rearrangements in the *BRCA1* mutation carriers, versus only 3.57% (0.76) in the control samples, $p=0.0001$. This provides new evidence that heterozygous mutation carriers have a different response to DNA damage compared with non-carriers. Our finding has implications for treatment and screening using modalities that involve irradiation in *BRCA1* mutation carriers. *joint first author.

Identification of genes targeted by ETV6-AML1 chimeric transcription factor. *S. Langlois, G. Boily, J. Larose, D. Sinnott.* Division of Hematology-Oncology, Research Center, Sainte-Justine Hospital, University of Montreal, Canada.

Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer. Moreover, chromosomal abnormalities, such as translocations, are often present in leukemia. The translocation t(12;21) is observed in approximately 25 % of children with pre-B ALL. The resulting chimeric protein, ETV6-AML1, consists of the DNA binding domain of AML1 and the repression domain of ETV6. Very little is known about the implication of ETV6-AML1 in leukemogenesis. We propose that this chimeric transcription factor acts in such a way that it represses transcription of genes usually activated by AML1. The principal objective of this study is to identify target genes of ETV6-AML1, in order to understand the role of this chimeric protein in leukemogenesis. Toward this goal, we have developed a HeLa cellular model, in which ETV6-AML1 expression is under the control of an inducible system (TetON). The mRNA obtained at different ETV6-AML1 induction times (0h, 4h, 12h, 48h) has been hybridized to the HG-U133A (22 000 genes) Affymetrix GeneChip, in order to detect the impact of ETV6-AML1 induction on the global expression profile. Initial analysis of the data led to the identification of 47 potential target genes. The expression of 14 of these candidate genes has been validated by quantitative real-time PCR. Then, we determined the expression profile of these 14 genes in 17 ALL patients with or without the translocation t(12;21), in order to evaluate the behavior of these targets in leukemia in respect to ETV6-AML1 expression status. Preliminary results show a nearly significant difference of expression for JUN, that is less expressed in patients with the translocation. Other putative target genes are being investigated. The promoter region of genes showing a correlation with the translocation in patients will eventually be studied using gene reporter assay, in the HeLa inducible model. Gel shift and super shift will also be carried out to determine whether the genes are direct targets of the ETV6-AML1 chimeric protein.

The human telomeric protein TRF2 is involved in a rapid cellular response to DNA damage. *P.S. Bradshaw¹, D.J. Stavropolous¹, M.S. Meyn^{1, 2, 3}.* 1) Genetics and Genomic Biology, Hospital for Sick Children, Toronto, ON; 2) Molecular and Medical Genetics, University of Toronto, Toronto, ON; 3) Paediatrics, University of Toronto, Toronto, ON.

Human cells use complex signaling networks to sense double-strand breaks (DSBs) in genomic DNA, then activate DNA repair, cell cycle checkpoints, and apoptosis. TRF2 prevents the ends of telomeres from triggering cellular DSB responses by complexing with telomeric DNA. We now report that TRF2 rapidly associates with laser-induced DSBs in non-telomeric DNA of human fibroblasts.

We find that TRF2 forms transient foci that tightly colocalize with DSBs and with DSB-associated foci of ATM, a protein kinase that controls multiple cellular DSB responses. Accumulation of TRF2 at induced DSBs occurs in the absence of functional ATM and DNA-PKcs protein kinases, the MRE11/Rad50/NBS1 complex or the Ku70, WRN and BLM repair proteins. This TRF2 response does not require expression of telomerase, as it occurs in both transformed and primary fibroblasts. The kinetics of YFP-TRF2 accumulation at DSBs were faster than those of GFP-ATM, YFP-NBS1 and of H2AX, a phosphorylated histone thought to be involved in recruiting repair proteins to DSB-containing chromatin. Deletion mapping indicates that the amino-terminal basic domain is required for TRF2 to associate with DSBs, the first known function for this domain.

These observations suggested that TRF2 is temporally and spatially poised to affect DSB activation of ATM damage responses. To test this, we over-expressed TRF2 in primary human fibroblasts. TRF2 over-expression resulted in reduced accumulation of phosphorylated ATM, H2AX and p53 following irradiation.

This work documents, for the first time, a non-telomeric function for TRF2. Our results suggest that TRF2 may interact with DSB-containing chromatin, provide evidence that TRF2 can compete with or attenuate ATM responses to DSBs, and implicate TRF2 in an ATM-independent step in DSB recognition/processing that occurs prior to the association of ATM with DSBs and activation of the ATM-dependent DSB response network.

The unusually high incidence of Germline Arg337His mutation in the p53 Tumor Suppressor gene among Brazilian Patients with adrenocortical tumors can be attributed to Founder Effect. *E.M. Pinto, A.E.C. Billerbeck, B.B. Mendonca, A.C. Latronico.* Endocrinology, Sch Medicine - Univ. Sao Paulo, Sao Paulo, Brazil.

An inherited missense mutation of the p53 tumor suppressor gene (Arg337His) was identified in high frequency (78-97%) in Brazilian children with benign and malignant adrenocortical tumors. Given the high frequency of this germline mutation in Brazilian population with adrenocortical tumor, it is most likely that the Arg337His mutation arose from a single founder. In this study, we analyzed two intragenic highly polymorphic markers (VNTRp53 and p53CA) in 22 apparently unrelated patients with adrenocortical tumors associated with the germline Arg337His mutation and in 60 normal individuals. The region enclosed each intragenic microsatellite were amplified by PCR and analysed by GeneScan Fragment Analysis Software. We found six and fourteen different alleles for the VNTRp53 and p53CA polymorphic markers, respectively. One allele for each marker, both with 122 bp fragment size, were found in 54.5% of the 44 alleles from patients with adrenocortical tumors. In contrast, these VNTRp53 and p53CA alleles were found in 18% and 14% of 120 alleles from normal controls, respectively ($p < 0.01$, chi square test). An identical haplotype for p53 locus was identified in 86% of the apparently unrelated Brazilian patients with adrenocortical tumors carrying the Arg337His mutation. These findings contrast with a previous study that eliminated Founder Effect in 17 unrelated patients with adrenocortical tumors and the Arg337His mutation. In conclusion, we demonstrated a strong evidence of co-segregation between two intragenic polymorphic markers and the germline Arg337His mutation of p53 tumor suppressor. The significant distribution of marker allele frequencies between patients with adrenocortical tumors carrying the Arg337His mutation and normal population indicated that the Arg337His mutation has a single common ancestral in the Brazilian population.

Common fragile site genes and neurological development. *D.I Smith, S. McAvoy, B.E. Armstrong, F. Wang.* Div of Experimental Pathology, Mayo Clinic College of Medicine, Rochester, MN.

The common fragile sites (CFSs) are large unstable genomic regions present in all individuals. Most of the research on the CFSs has focused on their role in promoting genome alterations such as translocations, deletions and gene amplifications in cancer cells. Of the 90 described CFSs, only 9 have been precisely defined at the molecular level. In these characterized CFSs, the regions of instability range in size from 500Kb (FRA7G) to greater than 9Mb (FRA9E). Overall, the nine defined CFSs encompass 23 Mbs of genomic sequence containing 55 interesting genes. These include a number of small genes, such as ARH1, caveolin-1 and IGF2-R that are known to play important roles in the development of certain types of cancer. There are also very large genes such as FHIT (1.5Mbs), LRP1B (1.9 Mbs), WWOX (1.0 Mb) and Parkin (1.4 Mbs) whose roles in cancer development are just being elucidated. CFS genes have also been associated with a number of neurological disorders, including Alzheimers disease, Autosomal Recessive Juvenile Parkinsonism, autism, and several of the ataxias. The 135Kb spinocerebellar ataxia, SCA7, gene was previously located within the centromeric portion of FRA3B. We now demonstrate that SCA1 (476 Kb) is a large conserved CFS gene spanning the FRA6C (6p22.2) CFS. Both SCA1 and SCA7 contain unstable trinucleotide repeats encoding polyglutamine tracts. Non CGG-repeats have not previously been associated with any type of chromosomal instability. We examined each of the unstable trinucleotide repeats associated with disease and found that a number of these are contained within relatively large genes mapping to chromosomal bands known to contain a CFS. Many genes containing unstable trinucleotide repeats are associated with neurological disorders, thus this provides further support that CFS genes play important roles in neurological development. The presence of these important neurological genes in highly unstable CFS regions suggests that they are probably highly susceptible to alterations during cancer development . There may also be an important connection between normal neurological development and changes observed in developing cancer cells.

Chromosome 12, Frequently Deleted in Human Pancreatic Cancer, May Encode a Tumor Suppressor Gene that Suppresses Angiogenesis. *S. Yamanaka*^{1,3}, *M. Sunamura*², *T. Furukawa*¹, *L. Sun*², *L.P. Lefter*^{1,2}, *T. Abe*^{1,2}, *T. Yatsuoka*^{1,2}, *H. Fijimura*², *E. Shibuya*², *N. Kotobuki*⁴, *M. Oshimura*⁴, *A. Sakurada*³, *M. Sato*³, *T. Kondo*³, *S. Matsuno*², *A. Horii*¹. 1) Dept Mol Pathol, Tohoku Univ Sch Med, Sendai, Japan; 2) Dept Gastroentero Surg, Tohoku Univ Sch Med; 3) Dept Thorac Surg, IDAC, Tohoku Univ; 4) Dept Cell Tech, Tottori Univ, Yonago, Japan.

Several lines of evidence have suggested that the long arm of chromosome 12 may carry a tumor suppressor gene(s) that plays a role in pancreatic ductal carcinogenesis. We have previously found a significant association between LOH of the 12q arm and a poor prognosis in pancreatic cancer patients. In this study, we introduced a normal copy of chromosome 12 into some pancreatic ductal carcinoma cells. Both anchorage-dependent and -independent proliferations as well as invasiveness were similar throughout the hybrid clones when compared with their corresponding parental cells. In sharp contrast, significant suppression of tumorigenesis was observed after inoculation of the hybrid clones into nude mice. Measurements made up to one month later showed that there was a significant delay in the growth of tumors into which the introduced normal copy of chromosome 12 had been restored. More significantly, using our dorsal skin chamber and an intravital microscopy system experiment in SCID mice, we demonstrated and visualized directly that implantation of the hybrids failed to promote the angiogenic phenotype encountered in the parental cells. Gene expression profiling using the cDNA microarray system identified a set of 24 genes differentially expressed between the hybrids and parental cells. An additional set of 18 genes was also identified that were differentially expressed between the hybrid clone that lost its growth suppression activity and one that retained such activity. Another set of 25 genes mapped on 12q was detected that showed high expression levels in the hybrid clones retaining growth suppressive activity. In summary, this study provides the first functional evidence of the existence of an additional tumor suppressor gene(s) on chromosome 12 whose absence is responsible for the pathogenesis in pancreatic ductal carcinogenesis.

Non Hodgking Lymphoma: incidence of Bcl-2/IgH rearrangements in Mexican patients and its relevance as marker for minimal residual disease. *R. Arana*^{1, 2}, *G. Ignacio*³, *S. Baltazar*³, *M. Rubio*³, *E. Baez*³, *J. Duque*³, *F. Tripp*³, *S. Rivas*³, *O. Garces*³, *L. Solis*³, *V. Moran*⁴, *S. Kofman*^{1, 2}. 1) Genetics, Hospital General de Mexico, Mexico City, D.F., Mexico; 2) Facultad de Medicina, UNAM; 3) Grupo Multicentrico Mexicano de Hematologia; 4) Escuela de Medicina, Universidad Panamericana, Mexico.

The follicular lymphoma is characterized by the t(14;18)(q32;q21), which joins JH segments of the heavy chains of immunoglobulins (IgH) gene with the Bcl-2 gene. However, this translocation is also present in the non Hodgking lymphomas (NHL) of intermedium and high grade in 15 to 30% of cases. **OBJETIVE:** To determine the incidence of the Bcl2-IgH rearrangement in Mexican patients with follicular lymphoma and with NHL of intermedium and high grade. **PATIENTS AND METHODS:** 292 patients (151 male and 141 female) were evaluated with the following diagnosis: 152 had low grade lymphoma, 113 intermedium to high grade lymphoma and in 27 the grade was not determined. The analysis was made in blood samples (BS) in 64 cases (24%) or bone marrow (BM) in 228 (76%). Genomic DNA was obtained and the Bcl-2/IgH rearrangement was amplified by both PCR and nested PCR using primers for JH and exon-intron 3 region of Bcl-2 (MBR and MCR regions). In 135 out of 292 patients in clinical remission, the Bcl-2/IgH rearrangement was used as marker to determine minimal residual disease stage in cases ranging from 8 to 32 months of evolution. The incidence and tissue type analysis was compared using chi-square statistics. **RESULTS AND DISCUSSION:** In the low grade follicular NHL the Bcl-2/IgH rearrangement was positive in 79% of the cases with a breakage in the MBR region in 115 cases and in MCR in only 5 cases. In the intermedium to high grade NHL cases it was present in 33%; with a breakage in 35 cases in MBR y 2 in MCR. Regarding the tissue type (BS or BM) it was positive in 86% of cases in BM and in 42% in BS, with $p < 0.0001$. The frequency of Bcl-2/IgH rearrangement in cases of follicular lymphoma in Mexican patients was high, highlighting the relevance of the molecular analysis in the diagnosis and its use as a marker for minimal residual disease cases..

Testicular seminoma and DAZ microdeletion. *T. Boudawara*¹, *N.B. Abdelmoula*², *L. Keskes*², *T. Bienvenu*³, *A. Amouri*⁴, *A. Bahloul*⁵, *T. Rebai*². 1) Laboratoire d'Anapath, Hopital Habib Bourguiba, Sfax, Tunisia; 2) Laboratoire d'Histologie Faculte de Mdecine de Sfax, Tunisia; 3) Laboratoire de Biochimie et de Gntique Moleculaire Hopital de Cochin Paris, France; 4) Laboratoire de Cytogenetique Institut Pasteur de Tunis, Tunisia; 5) Service d'Urologie, Hopital Habib Bourguiba, Sfax, Tunisia.

Testicular germ cell cancer is etiologically linked to genital malformations and male infertility and is most probably caused by a disruption of embryonic programming and gonadal development during fetal life. In some cases, germ cell neoplasia is associated with a relative reduction of Y chromosomal material or other abnormalities of the Y chromosome which contains genes critical for germ cell differentiation and spermatogenesis. Here we report a 33 years old man who complained of primary infertility of 5 years. He was found azoospermic by seminal analysis according to WHO guidelines. Physical examination, hormonal investigations and testicular ultrasonography were with normal findings. Bilateral testicular biopsy established the diagnosis of in situ seminoma at the right side and showed lack of all germ cells in both testes and presence of an abundant proliferation of Leydig cells. After genetic counseling, the patient's karyotype, analyzed using standard cytogenetic methods, was normal: 46,XY but analysis of his genomic DNA, tested using PCR-denaturing gradient gel electrophoresis method, showed microdeletion of the DAZ gene. The patient was member of a very large family characterized by multiple men with infertility (three brothers and two cousins). Only one cousin who was oligospermic has been genetically investigated and found deleted of the DAZ gene. It has been suggested that DAZ microdeletions, in addition to causing infertility with a variable phenotypic expression, predispose also to germ cell neoplasia. We will discuss through this observation, vertical transmission of Yq microdeletions and the susceptibility of DAZ microdeleted men to develop testicular tumors.

Molecular profiling of oral cancer using DD-PCR and microarray techniques. *S. Chakraborty*¹, *S. Swamy*², *A. Moinuddin*³, *K.S. Gopinath*², *A. Kumar*¹. 1) MRDG, Indian Institute of Science, Bangalore, Karnataka, India; 2) Bangalore Institute of Oncology, Bangalore, India; 3) R.L. Jalappa Hospital and Research Center, Kolar, India.

Oral squamous cell carcinoma (OSCC) is the sixth most common malignancy and a major cause of cancer morbidity and mortality worldwide. In India, it is the leading cancer in males and the third most common in females, due to the widespread habits of tobacco chewing and smoking in conjunction with alcohol consumption. Although the risk factors for oral cancer are well established, its clear cut molecular mechanism remains elusive. The overall five year survival period of OSCC is among the lowest for all major cancers which has not changed in the past two decades. Thus finding a biological tumor marker to increase the early diagnosis and treatment is of paramount importance. Given the high incidence of oral cancer in the Indian population, we have set out to elucidate the genetic and biochemical alterations that underlie its onset and progression. In order to obtain a tissue specific gene expression profile, we have employed the DD-PCR and microarray techniques. The DD-PCR profiling showed 51 genes which were either upregulated or downregulated in OSCC. Northern and Reverse Northern analyses revealed that 31/51 genes were truly differential. Of 31 genes, one upregulated and two downregulated genes were validated by Semi-quantitative RT-PCR in a panel of 16 matched normal and tumor samples. We are in the process of sequencing and validating rest of the genes. To obtain a global profile of the molecular changes inherent in OSCC, cDNA microarray analysis was carried out, using three paired samples of clinically confirmed cases on human 19k gene arrays. The study revealed more than 300 genes to be deregulated which are known to be involved in a variety of cellular functions. We are in the process of validating the microarray data. (Financial support from the CSIR and ICMR is gratefully acknowledged).

Clinical prognosis of breast cancer patients with ATM missense mutations. *J. Feng¹, A. Singh³, M. Jung³, C. Buzin¹, J. Moulds³, Y. Zhang², E. Gehan², A. Dritschilo³, S. Sommer¹.* 1) Dept Molecular Genetics, City of Hope Natl Medical Ctr, Duarte, CA; 2) Dept Biostatistics, Lombardi Cancer Center, Washington, DC; 3) Dept Radiation Medicine, Georgetown University Medical Center, Washington, DC.

Ataxia-Telangiectasia (A-T) is an autosomal recessive disorder characterized by progressive cerebellar degeneration, telangiectasias, radiosensitivity, immunodeficiency, and a predisposition to cancer development. Epidemiologic studies of families of A-T patients have demonstrated an increased risk of breast cancer in females. Furthermore, an elevated frequency of missense ATM gene mutations has been observed in patients with sporadic breast cancer. In a recently reported series, we observed a significant elevation of cohort-specific mutations in 90 breast cancer patients as compared to ethnically matched controls (relative risk: 3.2; attributable risk: 13%). Subsequently we have retrospectively reviewed the records of breast cancer cases for correlation with clinical features. The 11 year disease-free survival was 43% and 93% for patients with and without mutations respectively ($p=0.001$), suggesting that ATM mutations confer a negative clinical prognosis in breast cancer.

Germline in-frame deletion of exons 18-22 in retinoblastoma. *K. Heimdal¹, L. Steele², P.A. Andresen³, R.S. Tranheim⁴, L. Walaas³, P.N. Ray², A. Heiberg¹, N.A. Eide⁴.* 1) Dept Medical Genetics, Rikshospitalet, Oslo, Norway; 2) Molecular Genetics Laboratory, Hospital for Sick Children, Toronto, ON, Canada; 3) Dept Pathology, Rikshospitalet, Oslo, Norway; 4) Dept Ophthalmology, Ullevål University Hospital, Oslo, Norway.

Identification of RB1 mutations is useful in genetic counseling. Generally, the penetrance of RB1 mutations is very high (>95%), however, low-penetrance families have been described. We report the results of molecular findings in a family where a 19-year-old female developed unilateral retinoblastoma. The tumour presented as uveitis and the diagnosis was made by demonstration of calcifications in the tumour and retinoblastoma cells in a fine needle aspirate. A second cousin on the paternal side had unilateral retinoblastoma at 2 years. The tumour was treated with chemotherapy and local and external radiotherapy. The patient is free of disease at 6 years. Testing by direct mutation analyses of DNA from peripheral blood demonstrated a heterozygous deletion of exons 18-22 of the RB1 gene. Deletion analysis with FISH for chromosome region 13q14 was normal. DNA from the cytology specimen was not available for molecular testing. The mutation was not present in the affected paternal second cousin but was inherited from the maternal side. The results of direct mutation analysis have been confirmed by haplotyping using flanking and intragenic markers. The mutation has not been described previously. We believe this is an RB1 mutation with reduced penetrance. The mutation is predicted to lead to an in-frame deletion and a partially dysfunctioning retinoblastoma protein. In-frame deletion of exons 24 and 25 has been reported previously in a large Chinese retinoblastoma family showing markedly reduced penetrance. Further studies of the mutation may give useful information about RB1 function. The use of mutation data in genetic counselling in this family is complicated due to limited information on penetrance and expression. So far, known mutation carriers have not developed malignancies other than retinoblastoma.

Role the androgen receptor in germline susceptibility to prostate cancer. *M. Zeegers¹, A. Nieder², L. Kiemeny³, H. Ostrer⁴*. 1) University of Birmingham, UK; 2) University of Miami, FL; 3) University Medical Centre Nijmegen, NL; 4) New York University School of Medicine, NY.

Many published studies suggested an association between (CAG)_n and (GGN)_n polymorphisms in the AR gene and prostate cancer. To quantify this association, we performed a meta-analysis to provide both relative and absolute quantitative summary estimates with sufficient power. Publications were identified through database and hand searches for epidemiologic studies published until February, 2004. For each study, we calculated mean differences in repeat length between cases and controls, continuous odds ratios (OR_c) per one CAG or GGN repeat decrement and discrete odds ratios (OR_d) to compare prostate cancer risk in men with short CAG repeats (21 repeats) versus long CAG repeats (>21 repeats) and short GGN repeats (16 repeats) versus long GGN repeats (>16). The study specific estimates were combined by random effects meta-regression analyses. 19 case-control studies were included in this review comprising a total of 4,274 cases and 5,275 controls. Prostate cancer cases had on average 0.26 fewer CAG repeats and 0.09 fewer GGN repeats than controls. The OR_c of prostate cancer per 1 repeat decrement were 1.02 and 1.01 for CAG and GGN repeats, respectively. The summary OR_d were 1.19 (95th centile CI=1.07-1.31) and 1.31 (95th centile CI=1.06-1.61), for CAG and GGN repeat polymorphisms, respectively. Although the presence of shorter repeats appeared to be modestly associated with prostate cancer risk, the absolute difference in number of repeats between cases and controls is less than one repeat.

Genetics and Cancer Research: an overview 1985-2004. *L. Bouchard*¹, *R. Dalpe*², *M. Sampson*³. 1) Sociology/Inst Pop Health, University of Ottawa, Ottawa, ON, Canada; 2) Political Sciences, University of Montreal, Montreal, QC; 3) Chalmers Research Group, Children Hospital Research Institute, Ottawa, ON.

Aim:To trace the evolution of the genetic & common components of cancer research, specially breast(B)& colorectal(C)Cancer since 1985, through a bibliometric study.**Method:**We use previously validated keywords to identify scientific publications and exploiting OVID Medline indexing features, classified them as systematic reviews, clinical trial, commentaries and guidelines. We compare their number for all Ca, B&C Ca, Genetic B&C. **Results:** Since 1985, about 1 million articles have been indexed on all Ca; 250,000 on B&C; 60,000 on these Cancers' genetic. Breast Ca papers accounted for 10% of the whole. **Preclin. res.:** 170,000 for all; 38,000 for B&C; 12,000 for Genetic. **Syst. rev.:** 7000 for all; 2000 for B&C; 500 for Genetic. **Guidelines:** 2000 for all; 500 for B&C, 100 for Genetic. **Comments:** 4000 for all; 1000 for B&C; 200 for Genetic. 60-70% of genetic papers related to breast Ca. Scientific production of genetic research on B&C Ca counts 5000 publications from 1985-89 and over 20,000 during 2000-04. The 5 cancer journals with the highest impact were: CA J Clin, J Natl Inst, Nat Rev CA, J Clin Oncol, Adv CA Res. These 5 journals account for 2% of preclin. studies in the genetic Ca; 8,7% of clin. trials; 9,6% of guidelines; 21,1% of comments. **Discussion:** 25% of Ca research has concentrated on B&C Ca, a fifth including a genetic component. We saw a growth of scientific publications since 1990 in genetic research, notably in clin. trials, syst. rev., guidelines and comments. Production has doubled between 1990-94 & 95-99, although this growth has slowed down since 2000. The Ca journals with the HI factors represent the translation of research to practice, with a concentration of clin. trials, treatment guidelines and comments. A bibliometric study correlating the number of publications with the relative mean impact factor has explored features of scientific productivity and research performance. The search strategy used for C Ca or genetic Ca has not been published before and requires further refinement. Precision of bibliographic searches is rarely perfect and we relied on them for classification. Our results are convergent with others studies.

Mutation pattern in DNA binding domain of p53 gene in squamous-cell carcinoma of the esophagus in Kashmir (India), a high incidence area in the world. *S. Gochhait¹, M.M. Mir², N.A. Dar², S.A. Zargar³, A.G. Ahangar⁴, R.*

Bamezai¹. 1) NCAHG, SLS, Jawaharlal Nehru University, New Delhi, Delhi, India; 2) Department of Clinical Biochemistry, Sher-I-Kashmir Institute of Medical Sciences, Soura, Srinagar, J&K, India; 3) Department of Gastroenterology, Sher-I-Kashmir Institute of Medical Sciences, Soura, Srinagar, J&K, India; 4) Department of Cardiovascular Thoracic Surgery, Sher-I-Kashmir Institute of Medical Sciences, Soura, Srinagar, J&K, India.

A high incidence of esophageal cancer in Kashmir valley (India) has been attributed to an exposure to nitroso compounds (or their precursors), amines and nitrates present in the staple food, hot salted tea (Noon Chai) and the pipe (Hukka) smoke. However, till date, no attempt has been made to study the presence of mutations in tumours of patients with esophageal cancer from Kashmir valley, following a distinct life style and dietary habits. Here, we make a preliminary attempt to study esophageal squamous cell carcinoma (ESCC) tissues of 55 patients from Kashmir valley for mutations in exons 5-8 (the DNA binding domain) of a tumour suppressor gene, p53. Direct sequencing analysis revealed the presence of mutation in 36.36% tumours. Of the 20 mutations in 20 patients, 17 were single base substitutions (11 transitions + 6 transversion) and 3 deletions. Seventeen single base variations represented 12 missense mutations leading to amino acid substitution, 2 nonsense mutations and 3 variations located in intron 6, one of which resulted in a splicing variant. There was a high prevalence of mutation in exon 5 (65%) and surprisingly an absence of mutation in exon 8. The frequency of mutation when compared between males (25%) and females (57.89%) turned out to be significant ($p=0.016$), apparently putting females at a higher risk of carrying p53 mutation. The mutation pattern (when compared to IARC database) suggested that both the sexes have differential proneness to the induction of mutation by the exposure to carcinogens, to which they are equally exposed through diet and life style, an observation, which would need a further study of a larger set of samples from Kashmiri population.

Differentially expressed genes identified by comparison of mRNAs from an adrenocortical cell line (NCI-H295A) and normal human adrenal. *M.N. Gouvea¹, A.S. Barbosa², B.B. Mendonca², C.J. Lin².* 1) Dept Patologia, Fac Medicina, Univ Sao Paulo, Sao Paulo, Brazil; 2) Lab Hormonios e Genetica Molecular - LIM/42 - Hosp Clinicas - Fac Medicina, Univ Sao Paulo - SP - Brazil.

Adenocarcinoma of adrenal cortex is a rare pathological condition accounting for 0.05% - 0.2% of all cancers. These tumors are usually associated with unfavorable prognosis. Distinction between localized malignant tumors and benign adrenocortical neoplasms, based on clinical and radiological data, can be difficult. Several genetic, molecular and imunohistochemical markers have been evaluated for their usefulness in outcome prediction. However, none has been shown to reliably distinguish carcinomas from adenomas. The aim of this study is to identify genes differentially expressed in malignant adrenocortical tumors. Using the mRNA differential display we compared mRNAs isolated from NCI-H295A cells - a steroid-producing cell line derived from an invasive primary adrenocortical neoplasm with a commercially available RNA prepared using a pool of normal human adrenal. In NCI-H295A cells we observed overexpression of transcripts involved in important biological processes such as regulation of cell cycle (ERH), transcription regulation (VEGFA), maintainance of cell function (SNAP91), peroxisome organization and biogenesis (PAF1). In opposition, in normal adrenal we found higher abundance of transcripts for genes related to cell cycle arrest (AFX1), mitogenesis and differentiation (EGR-1), cytoskeleton and cell motility (DNAI1), cholesterol biosynthesis (DHCR24), and protein biosynthesis (RPL27A). In conclusion, our preliminary comparison of transcriptomes revealed substantial differences between normal adrenals and a cell line derived from an adrenocortical carcinoma. These differentially expressed transcripts will be further validated in tumoral samples in order to assess their roles in tumorigenesis and in the prediction of tumoral outcome. Interestingly, approximately 10% of our transcripts showed no homology to known genes deposited in public sequence databases. Such transcripts might represent genes not described so far and might have a role in the tumorigenesis of adrenal cortex.

A new treatment to improve the treatment of women with ER-negative/ PR-negative breast cancer. *S. Toth-Fejel, C. Hardin, P. Muller, T. Jackson, R. Pommier.* Surgery, Division Surgical Oncology, Oregon Health Sci Univ, Portland, OR.

We have shown that 228 uM dehydroepiandrosterone-sulfate (DHEAS) plus an aromatase inhibitor resulted in significant cell death in four estrogen receptor negative and progesterone receptor negative, but androgen receptor positive (ER negative/PR negative/AR positive) breast cancer cell lines (22% in HCC 1937 cells, 30.6 % in HCC 38 cells, 69% in HCC 1143 and 54 % in HCC 1954 cells. The inhibitory effect of DHEAS on these cells was nullified by the addition of the AR antagonist Casodex. For example, when these cells were treated with DHEAS, aromatase inhibitor and Casodex, the absolute percentage of cells undergoing cell death (apoptosis) decreased in both cell lines from 54.5% to 27.7% in the HCC 1143 cells and from 63 % to 22.8 % in HCC 1937 cells. These results implicate the AR as responsible for regulating of the cell death process. Preliminary immunoblotting data for the expression of apoptosis intermediaries has been completed showing that cell lines HCC 1143 and HCC 1954 increase in TRAIL and BAX expression above untreated cultures, when normalized to B-actin levels following 72 hours of DHEAS treatment. Of clinical relevance, within our breast cancer patient data base, 44% of ER negative patients are AR positive, indicating that there may be wide applicability of this treatment.

Linkage Analysis of Hereditary Prostate Cancer (HPC) to Xq27-28 in the ICPCG families. *J. Bailey-Wilson*¹, *B. Chang*², *J. Xu*², *International Consortium for Prostate Cancer Genetics*. 1) Inherited Disease Research Branch, NIH/NHGRI, Baltimore, MD; 2) Center for Human Genomics, Wake Forest University School of Medicine, Winston-Salem, NC.

The *HPCX* locus at Xq27-28 was first reported in a combined data set of 360 HPC families from North America, Sweden and Finland (Xu et al., 1998) but replication has been inconsistent. The purpose of the International Consortium for Prostate Cancer Genetics (ICPCG), an international collaborative effort to pool pedigrees with hereditary prostate cancer, is to replicate linkage findings for prostate cancer in a highly-powered dataset. Using a method we developed to combine multiple studies that used different genetic markers, a consensus map of the Xq region incorporating these markers was generated based on the UCSC physical map. The genetic distance of the markers was based on the deCode map. Each individual group then performed parametric and non-parametric linkage analyses at each CentiMorgan (cM) along the consensus map using the GENEHUNTER-PLUS computer program, and submitted the family-specific linkage results at each cM to the Data Coordinating Center. We then estimated overall parametric heterogeneity LOD scores using Smiths A-test and the compound LOD of Vieland et al. (2001) and non-parametric allele-sharing LOD scores using the ASM program. Eleven independent sets of data for a total of 1260 prostate cancer families were used. The maximum multipoint HLOD and compound LOD obtained from families not included in the original linkage report were 0.82 and 1.67 near DXS984 at 145.8 cM; using all families these scores were 1.57 and 2.65 near DXS8091 at 167.3 cM. The non-parametric multipoint allele-sharing LODs were 1.32 near DXS984 and 2.0 near DXS1227 at 150.37 cM in the new and all families, respectively. Subset analyses taking account of age at diagnosis, compatibility with X-linked inheritance, etc. are ongoing. The linkage peaks were broad and support the presence of an HPC locus at Xq27-28, indicating the utility of the ICPCG data resource. (Supported by PHS U01-CA89600).

Screen for prostate cancer susceptibility genes in 1,139 families: ICPCG combined genome-wide screen. *J. Xu, The International Consortium for Prostate Cancer Genetics. Ctr Human Genomics, Wake Forest Univ Sch of Med, Winston Salem, NC.*

Evidence for the existence of major prostate cancer susceptibility genes has been provided by multiple segregation analyses. Genome-wide screens for such major susceptibility genes have been performed in over ten independent study populations. However, few chromosomal regions have been consistently identified and replicated across these different studies. This may be expected considering the complexity of familial prostate cancer, resulting from factors such as genetic heterogeneity, incomplete penetrance, and a high phenocopy rate due to the extremely high prevalence of this disease. As a means to increase power to detect major prostate cancer genes, we combined linkage data available for the large number of prostate cancer families collected by the International Consortium for Prostate Cancer Genetics (ICPCG), an international cooperative study involving over 75 investigators from 19 institutions. Using a method we developed to combine multiple individual genome-wide screen studies performed using different genetic markers, a consensus map incorporating these markers was generated based on the UCSC physical map. The genetic distance of the markers was based on deCode map. Each individual group then re-performed parametric and non-parametric linkage analyses at each CentiMorgan (cM) across the genome using the Genehunter computer program, and submitted the family-specific linkage results at each cM to the Data Coordinating Center (DCC). The DCC then estimated overall parametric linkage score using HOMOG and non-parametric linkage score using ASM. Nine independent studies, with a total of 1,139 prostate cancer families, were included for this combined analysis. Initial analyses of these combined data identified two regions with HLOD over 1.5 on chromosomes 2 and 11 in the complete set of families. Higher HLOD scores were observed in subsets of families, e.g. among the 578 families with family mean age at diagnosis less than 65, an HLOD of 2.66 was observed at Xq11 in close proximity to the AR gene. Further analyses of this extensive dataset incorporating additional family, ethnic and clinical variables are underway. (Supported by PHS U01-CA89600).

High incidence of hidden chromosomal aberrations detected by FISH profiles (TEL/AML1, p16, MLL, BCR/ABL) in acute lymphoblastic leukemia. *E.H. Cho¹, H.Y. Woo², S.G. Lee¹, S.Y. Kong¹, S.H. Kim¹.* 1) Department of Laboratory Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; 2) Department of Laboratory Medicine, Kangbuk Samsung Hospital, Seoul, Korea.

Cytogenetic analysis in ALL is often hampered by few malignant metaphases, poor morphology, cryptic chromosomal rearrangements and sometimes by only normal metaphases derived from normal cells. To find hidden aberrations, we have analyzed 64 ALL patients with normal karyotype[49] or no metastasis[15] using FISH profile tests (FISH for TEL/AML, p16 deletion, MLL rearrangement and BCR/ABL). 40 (70%) of 64 cases showed abnormal FISH results. The cases of abnormal signals were 27 for TEL/AML1, 21 for p16, 15 for MLL and 7 for BCR/ABL. Cases with intended rearrangement of tested FISH probes were 7 for TEL/AML1 translocations, 17 for p16 deletions (homozygous loss[9], hemizygous loss[8]), 7 for MLL break apart signals and 1 for BCR/ABL translocation. FISH studies also showed unintended abnormal signals involving the loci tested, which were additional copies of p16[2], MLL[3] and BCR[1]. FISH for TEL/AML1 showed additional copies of AML1[5], AML1 amplification[2] and loss of TEL[2]. 5 cases showed signal pattern suggesting massive hyperdiploid. FISH for TEL/AML1 was most informative among tested FISH probes (detection rate;42%) and showed variable abnormal signals. There were 10 cases with only abnormality in FISH for p16 deletion among FISH profile test, which suggest the addition of FISH p16 probe to other commonly used FISH profile tests in ALL could be highly informative. This study showed very high incidence of hidden chromosomal aberrations could be detected by FISH profiles in ALL cases with normal karyotype or no metastasis.

Prevalence and Prognostic Value of Microsatellite Instability in Sporadic Rectal Tumours. *R. Coura^{1, 2}, S.L. Cossio², J.C. Prolla³, M.A. Rosito³, L. Meurer³, P. Ashton Prolla^{1,2,4}.* 1) PPG Genetica e Biologia Molecular, UFRGS, Porto Alegre, RS, Brazil; 2) Servio de Gentica Mdica, HCPA, Porto Alegre, RS, Brazil; 3) HCPA, Porto Alegre, RS, Brazil; 4) Departamento de Gentica, UFRGS, Porto Alegre, RS, Brazil.

Colorectal cancer (CRC) is the third tumor in frequency and the second in mortality in developed countries. In Brazil, it is one of the six most common cancers and the third in mortality. Among all cases of CRC, about 85% are sporadic. Prognostic evaluation is currently based only on clinical-pathological and morphological parameters, since the prognostic value of molecular markers requires further elucidation. Mutations in mismatch repair genes (MMR) are associated with hereditary CRC (particularly HNPCC), but they can also be observed in some cases of sporadic CRC. These MMR mutations (in hMSH2 and hMLH1, especially) result in a replication error phenotype, which consequently generates microsatellite instability (MSI). MSI frequency in HNPCC is more than 90% and, in sporadic CRC it is about 15%. Tumours with MSI tend to present better prognosis: greater survival rates, minor metastasis occurrence and greater response to certain chemotherapeutic regimens. In this study we evaluate the prevalence and prognostic value of a panel of five markers for MSI analysis in sporadic CRC. MSI was analysed by PCR and SSCP. The sample consisted of 42 patients with rectal cancer was completely analysed for two mononucleotide markers, BAT-25 and BAT-26. We found a MSI frequency of 7.1% and 7.5% for BAT-25 and BAT-26, respectively. Both markers were simultaneously unstable in 2.4% of patients. These results are in agreement with the literature that reports MSI frequency of less than 10% in sporadic CRC. Furthermore, about 7.7% and 15% of patients with CRC under the age of 55 showed BAT-25 and BAT-26 MSI, respectively. This corroborates with literature reports that point out the early age as a predictive factor of the presence of MSI. Three additional markers (D2S123, D5S346 and D17S250) are being analysed. Prognostic value and correlations with clinical-pathological and morphological parameters will be determined. Support: CNPq, CAPES, HCPA.

Identification of candidate genes in 6p22 that are targeted by amplification in the progression of retinoblastoma.

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Mutations in both alleles of the RB-gene are required for development of retinoblastoma (Rb), a childhood cancer of the eye. Additional genetic alterations occur during progression of this tumor. These include gains of genetic material on 1q31-q32 and on 6p22-p23. Recently, the minimal region of amplification on 6p was narrowed down to a region of 0.6 Mb containing the KIF13A, which codes for a motor protein. To identify other genes targeted by gains in this region we determined the relative gene dosage on 6p22.3 in 78 Rb by CGH and quantitative Multiplex PCR (QM-PCR). Microarray Analysis was used to investigate RNA expression in 23 tumors. QM-PCR analysis included 14 loci in a 2 Mb region on 6p22.3 - 21. Relative gene dosage was calculated based on two control loci on 6q and 10 q. Results obtained with normal controls (peripheral blood DNA) were used for normalization. We found that 38 of 78 Rb showed unaltered gene dosage for all loci. In 24 of 78 samples an increased gene dosage was identified for all loci on chromosome 6p, a finding consistent with the presence of an isochromosome 6p. In 16 tumors, gains were restricted to some of the 6p-loci. QM-PCR data were positively correlated with CGH results in 47 Rb ($r = 0.8$). The minimal region of gain centered at 6p22.3. Gains were most pronounced for the DEK and E2F3 gene. RNA expression was analyzed in 23 Rb with Affymetrix CHIP U133A. In all these samples, KIF13A RNA was low or absent ($n=3$). For the other analyzed genes on 6p RNA expression values were positively correlated ($r = 0.78$) with DNA-dosage (QM-PCR). Significance Analysis of Microarrays (SAM) identified DEK, NUP153 and TTRAP as potential target genes of amplification on 6p. In summary, we have identified four genes, DEK, E2F3, TTRAP and NUP 153, as potential targets of increased gene dosage in 6p in Rb.

Systematic functional testing of the 3p21.3 critical region for tumour suppression of lung cancer. *A. ter Elst, B.E. Hiemstra, W. Kamming, I. Davelaar, A.Y. van der Veen, G.J. te Meerman, C.H.C.M. Buys.* Medical Genetics, University of Groningen, Groningen, Groningen, Netherlands.

The short arm of chromosome 3 shows allelic imbalances in 75-100% of lung tumours. This is seen as the first chromosomal change in lung tumour development. And is also found in 50% of histologically normal tissue of smokers and former-smokers, but not in controls. A critical region of 370 kb has been defined in the 3p21.3 region. This region contains 19 well-defined genes. We are systematically testing this region for tumour suppressor activity. We first introduce PACs from the critical region into a well-transfectable lung cancer cell line. We use PACs to make it likely that the genes are accompanied by their main regulatory sequences. We have isolated eight PACs overlapping a 440 kb homozygous deletion in the SCLC cell line GLC20, including the whole of the critical region. Stable transfectants are being checked for complete PAC integration, purity, single site of integration and absence of amplification. Transfectant cell clones meeting all criteria are then injected into nude mice for tumourigenicity studies. In a first experiment 24 mice have been injected subcutaneously on both sides. For one side we used two transfectant clones containing one and the same PAC from the centromeric part of the critical region, for the other side two transfectants each containing a different PAC from the middle part of the deletion. The tumours were carefully isolated, measured and weighed, four weeks after injection. The mean weight of the tumours caused by transfectants containing the centromeric PAC was significantly reduced in comparison with that of the tumours caused by transfectants containing PACs from the middle of the deletion. This PAC still contains six genes (RASSF1, BLU, NPR2L, PL6, 101F6 and CACNA2D2). Our result suggests that among these, is/are a gene/genes that are more important for tumour development than the genes GNAI2, SEMA3B, IFRD2, HYAL3 and NAT6, that are on the other PACs. Experiments with overlapping PACs may delimit the true critical region, although combined effects of several genes at 3p21.3 cannot be excluded.

***BRCA1* and *BRCA2* mutations in African and African American breast cancer patients.** J.D. Fackenthal¹, F. Hagos¹, H. Kim², B. Zhang¹, Q. Niu¹, C.A. Adebamowo³, T.O. Ogundiran³, A.A. Adenipekun³, R. Oyesegun³, O. Campbell³, E.U. Akang³, D. Fackenthal², S. Das², S. Neuhausen⁴, C. Rotimi⁵, O.I. Olopade¹. 1) Department of Medicine, University of Chicago, Chicago, IL; 2) Department of Human Genetics, University of Chicago, Chicago, IL; 3) University College Hospital, Ibadan, Nigeria; 4) Center for Cancer Genetics Research and Prevention, University of California Irvine, Irvine, CA; 5) National Human Genome Center at Howard University College of Medicine, Washington, DC.

Breast cancer in women of African descent strikes at an earlier average age and has a higher mortality rate than seen in other groups. To address whether this results from a distinctive hereditary risk, we identified the germline variants in the *BRCA1* and *BRCA2* genes in 57 African American and 38 Nigerian breast cancer patients using DHPLC and direct sequencing. Nine of the 95 (9.5%) carried protein-truncating *BRCA1* or *BRCA2* mutations, a potentially significant contribution considering patients were not selected by family history. In addition to the known deleterious mutations, this cohort carried a large number of unclassified variants and polymorphisms. In the African American subset, 39/57 (68.4%) and 47/57 (82.5%) of patients carried at least one variant in *BRCA1* or *BRCA2*, respectively. Likewise, in the Nigerian cohort, 23/38 (60.5%) and 31/38 (81.6%) of patients carried at least one variant in *BRCA1* or *BRCA2*, respectively. To address the potential clinical significance of these variants, we determined the genotypes of all patients along with 96 Nigerian control samples for 24 *BRCA1* or *BRCA2* variants by primer extension analysis. None of the variants showed statistically significant difference in variant frequencies between patients and controls. Taken together, we conclude that *BRCA1* and *BRCA2* mutations may play a significant role in breast cancer patients of African descent, yet much of the high genetic diversity of these genes reflects the genetic diversity of the ancestral populations rather than deleterious alleles particular to patient populations. Supported by NCIRO1 CA89085.

Lack of contribution of causative BRCA1 mutations to tumor phenotype in African American women. *L.R. Hoffman¹, R.E. Ellsworth¹, J. Hooke², C.D. Shriver².* 1) Clinical Breast Care Project, Windber Research Institute, Windber, PA; 2) Clinical Breast Care Project, Walter Reed Army Medical Center, Washington, DC.

Breast cancer displays different characteristics in African American women than in Caucasian women, including earlier onset and a less favorable clinical outcome. Biological characteristics of tumors in African American women such as early age of onset, and high-grade, larger and more aggressive tumors with lymph node involvement, and frequent negative estrogen receptor (ER) status, mimic tumors from women with BRCA1 mutations. Breast cancer specimens from African American women with invasive (stage I-IV) breast cancer (n = 20) enrolled in the Clinical Breast Care Project at Walter Reed Army Medical Center (an equal-access health care facility) were evaluated histologically for BRCA1-like characteristics, including high grade, high mitotic index, pushing tumor margins, and lymphocytic infiltrate. The entire coding region of the BRCA1 gene in these patients was examined by double-stranded sequencing and the results examined within the context of the Breast Cancer Information Core Database. Sequencing revealed a number of sequence variants, the majority within exon 11. These DNA changes include the unclassified variants H476R, previously seen only in Africans or African Americans; K820E, frequently seen in African and African Americans; and M1628V, not previously reported in African or African American populations. In addition to patients with invasive breast cancer, examination of the DNA from African American patients with ductal carcinoma in situ (n = 8) revealed the presence of BRCA1 polymorphisms in all patients, although no mutations, unclassified or otherwise, were detected. The absence of an association of causative BRCA1 mutations with the BRCA1-like phenotype suggests that, while BRCA1 mutations may be involved in breast cancer development in some African Americans, the contribution of BRCA1 is limited. Thus, genetic factors other than BRCA1 mutations likely exist that contribute to tumor etiology in African American women.

Standardization of assays for mutation screening of the BRCA1 gene amongst seven Ontario molecular genetics laboratories.

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The development of standardized assays and quality assurance surveys for large genes is complicated by the cost and complexity of mutation screening protocols. As a result some quality assurance testing programs have limited their challenges to assays of ethnic specific mutations only, which will not adequately address the entire testing protocol. In Ontario testing for BRCA1 gene mutations is provided to at risk individuals through seven provincial laboratories who have agreed upon testing protocols. The Ontario BRCA1 assay is based on the protein truncation test and sequencing of exons 2 and 5 of the BRCA1 gene. The seven laboratories agreed to participate in a quality assurance survey to determine if all were able to perform BRCA1 analysis to an equivalent standard. The laboratories were presented with 5 anonymized cases and given 12 weeks to complete their analyses. The cases included normal individuals and those with truncating mutations detectable through analysis of DNA or RNA. The cost to each laboratory of participating was \$5000 Cdn. Criteria for evaluation of the laboratory results and reports were established before being assessed by internal and external reviewers. The results of the study indicated that the laboratories were more similar in their abilities to produce an equivalent laboratory result than in the format of their reports. The study also exposed a limitation of the PTT test to detect mutations in the 5' end of BRCA1. Amongst its conclusions the study identified several options to consider for future surveys of large genes, they include developing a uniform template for the submission of laboratory data and reviewing a portion of a large gene on a rotating basis per challenge rather than the whole gene in a single challenge.

Development of a fluorescent multiplex assay for detection of MSI-High tumors. *J. Bacher¹, L. Flanagan¹, R. Smalley², N. Nassif¹, L. Burgart², R. Halberg¹, S. Thibodeau².* 1) Promega Corp, Madison, WI; 2) Mayo Clinic, Rochester, MN.

Determining whether a tumor exhibits MSI is useful for identifying patients with HNPCC and sporadic gastrointestinal cancers with defective DNA mismatch repair (MMR), aids in establishing a clinical prognosis and response to chemotherapy. A panel of markers was suggested for MSI analysis by a NCI workshop in 1997, but this panel has limitations resulting from the inclusion of dinucleotide markers that are less sensitive and specific for detection of tumors with MMR deficiencies compared to other types of markers. This study demonstrates that mononucleotides are the most sensitive and specific markers for detection of tumors with defects in MMR and identifies an optimal panel of markers for detection of MSI-High tumors. A set of 266 mono-, di-, tetra- and penta-nucleotide repeat microsatellite markers were used to screen for MSI in colorectal tumors. The best markers for detection of MSI-H tumors were selected for a MSI Analysis System, which includes five mononucleotide markers: BAT-25, BAT-26, NR-21, NR-24 and MONO-27. In addition, two pentanucleotide markers were added to identify sample mix-ups and/or contamination. We classified 153 colorectal tumors using the new MSI Analysis System and compared the results to those obtained with a panel of 10 microsatellite markers combined with IHC analysis. We observed 99% concordance between the two methods with nearly 100% accuracy in detection of MSI-H tumors. Approximately 5% of the MSI-H tumors had normal levels of four MMR proteins and as a result would have been misclassified based solely on IHC analysis, emphasizing the importance of performing MSI testing. The new MSI Analysis System offers several distinct advantages over other methods of MSI testing in that it is extremely sensitive and specific, amenable to high-throughput analysis, meets the 2004 NCI guidelines on MSI testing and overcomes problems inherent to the original NCI marker panel. The use of a single multiplex fluorescent MSI assay reduces the time and costs involved in MSI testing while increasing reliability and accuracy and thus should facilitate widespread screening for MSI in tumors of patients with gastrointestinal cancers.

VHL molecular testing: Real-Time PCR is an alternative quantitative method for the detection of large genomic rearrangements. A. Casarin¹, M. Martella¹, R. Polli¹, E. Leonardi¹, G. Opocher², A. Murgia¹. 1) Pediatrics, University of Padua, Padua, Italy; 2) Medical and Surgical Sciences, University of Padua, Italy.

Von Hippel-Lindau syndrome is an autosomal dominant familial cancer syndrome caused by mutations of the VHL gene (3p25). As a referral center for von Hippel-Lindau disease, we have analysed the VHL gene in a large series of unrelated individuals with clinically confirmed VHL or isolated VHL-related tumors. Disease-causing mutations have been detected in 55/55 of the clinical VHL individuals and in 9/123 of the monosymptomatic cases. A substantial fraction of these mutations (22/64; 34%) is represented by whole- or partial-gene deletions or by insertion/duplication rearrangements, not detectable by commonly used mutation scanning techniques. A comprehensive approach to the molecular analysis of VHL must therefore include a gene-dosage evaluation, currently obtained with a complex and time-consuming quantitative Southern blot analysis. With the aim of obtaining a reliable alternative strategy for the identification of large genomic rearrangements of the VHL gene we have developed a quantitative Real-Time PCR method. We have been able to set standard conditions for the analysis of each VHL exon, with the use of exonic primers and both TaqMan MGB and SYBR Green chemistries, on ABI Prism 7000 Sequence Detection System. The results we have obtained with this new method have consistently and reproducibly confirmed what found on normal controls and on deleted samples previously tested by quantitative Southern blot. The analysis of complex rearrangements is still underway and might need a precise characterization of the breakpoint regions. The precision and the high sensitivity of the Real-Time PCR represent clear advantages over previously applied techniques and could justify its use as a fast quantitative screening tool for VHL genetic testing. The possibility to determine the exact genomic dosage for each individual exon may allow a more precise mapping of the deleted regions and could be used to possibly refine the genotype-phenotype correlation in subjects with partial-gene deletions.

Abnormal expression of Period 1 (PER 1) in endometrial carcinoma. *J.G. Chang^{1,5}, K.T. Yeh², M.Y. Yang⁴, T.C. Liu⁴, W.L. Chan¹, R.C. Hsu³, P.H. Chou³, T.H. Chen³, S.F. Lin⁴.* 1) Dep Molec Med, China Medical University Hospi, Taichung, Taiwan; 2) Department of Pathology, Changhua Christian Hospital, Changhua, Taiwan; 3) Department of Obstetrics and Gynecology, Changhua Christian Hospital, Changhua, Taiwan; 4) Division of Hematology-Oncology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 5) Taipei Institute of Pathology, Taipei, Taiwan.

The development of endometrial carcinoma (EC) is a multiple-step process, which includes inactivation of tumor suppressor genes, activation of oncogenes, and disturbance of cancer-related genes. Recent studies have shown that the circadian cycle may influence cancer development and prognosis. The human circadian cycle is controlled by at least 8 circadian genes. In this study, we analyzed the expression of one circadian gene-PER1 in 35 EC and paired non-cancerous tissues by real-time quantitative reverse transcription polymerase chain reaction and immunohistochemical study. The results showed that the expression levels of PER1 in EC were significantly impaired. Mutational analysis of the whole gene and methylation analysis of cytosine-phosphate guanosine (CpG) sites at the promoter area were further performed to investigate the possible mechanisms. However, 4 polymorphisms were found in both cancerous and non-cancerous tissues, which had no relationship with the expression of PER1. In the promoter area of the PER1 gene, the CpG sites were methylated in 31.4% of EC, but in 11.4% of paired non-cancerous tissues ($P < 0.05$). Our results suggest that the down regulated PER1 expression in EC was partly due to the inactivation of the PER1 gene by methylation, and partly was inactivated by other unknown mechanism. We also analyzed the relationship among the expressions of PER1, P53, MYC, cyclin A, cyclin B and cyclin D1. We found that no definite relationship among them. Based on these results, we suggest that the down regulation of the PER1 gene may play a role in the development of some endometrial carcinomas, and it may result in disturbance of cell cycle.

Identification of novel mutations in *MYH* in North American patients demonstrates a requirement for whole-gene mutation screening. *K. Eliason*¹, *T. Judkins*¹, *B.C. Hendrickson*¹, *E. Lyon*², *M. Norton*¹, *V. Thompson*¹, *S. Gresko*¹, *B. Leclair*¹, *J. Barrus*¹, *S. David*³, *A. Livingston*³, *J. Reid*¹, *B.E. Ward*¹, *W.W. Noll*¹, *T. Scholl*¹. 1) Myriad Genetic Laboratories, Salt Lake City, UT; 2) Department of Pathology, University of Utah, Salt Lake City, UT; 3) Department of Chemistry, University of Utah, Salt Lake City, UT.

Germline mutations in the base excision repair gene *MYH* have recently been shown to confer susceptibility to colorectal adenomas and carcinomas. The most prevalent mutations in patients of British ancestry are G382D and Y165C. A set of 219 anonymous North American patient specimens submitted to confirm FAP diagnoses and negative for APC mutations were screened for Y165C and G382D. Thirteen instances of biallelic (5.9%) and 15 instances of heterozygous mutations (6.8%) were identified. Nine of the specimens with heterozygous mutations carried a second mutation after whole-gene sequencing, including two novel termination codons, E182X and Q300X, and a novel deletion mutation, IVS13+25del30. Several previously reported mutations were also detected. These results demonstrate a requirement for whole-gene screening as two thirds of the heterozygous patients carried a second mutation detected by sequencing. A further 100 of these specimens that were Y165C and G382D negative were evaluated by whole-gene sequencing. One instance of homozygous 1395delGGA, and one instance each of heterozygous mutations 1103delC and a novel splicing mutation IVS12-2AG were detected. Several novel missense variants of uncertain clinical significance were also identified.

A set of 306 anonymous patient samples negative for *MLH1* and *MSH2* clinical testing were screened for mutations in *MYH*. Thirteen (4.3%) samples had Y165C and/or G382D mutations, including one homozygous Y165C and two compound heterozygotes. One of the remaining ten heterozygotes was found also to carry 891+3AC. Sequencing 50 of these specimens that did not carry Y165C or G382D revealed an additional mutation, 1103delC. When compared with control specimens, those submitted for HNPCC testing show an overrepresentation of heterozygous *MYH* mutations (p0.05) and warrant further study.

Importance of confirming BCR/ABL fusion in the Diagnosis of Chronic Myeloid Leukemia. *M. Fang*¹, *D. Slater*², *J. Delach*¹, *P. Benn*¹. 1) Genetics & Developmental Biol, Univ Connecticut Health Ctr, Farmington, CT; 2) Backus Hospital, CT.

The presence of Philadelphia chromosome is a hallmark for the diagnosis of chronic myeloid leukemia (CML). Variant translocations are generally considered as bearing the same clinical course and outcome as simple t(9;22) translocations. In Ph-negative CML cases, BCR/ABL fusion gene or transcripts are usually detectable. We hereby report a cytogenetically ascertained Ph-positive case with typical pathological features of a myeloproliferative disorder consistent with CML. The patient received treatment with Hydrea, interferon, and Gleevec and achieved hematological remission but no cytogenetic remission at all for over seven years. Recently, molecular and molecular cytogenetic studies revealed absence of BCR/ABL fusion gene and transcripts. Furthermore, the Ph chromosome was found to be a derivative chromosome from a constitutional t(12;22) translocation instead of the variant t(9;22;12) translocation. This translocation was also inherited by the patient's son. Reevaluation of the patients clinical, hematological and morphological features in combination resulted in the diagnosis of idiopathic thrombocythemia. The patient subsequently received adjusted therapy and remains in hematological remission and in good clinical condition. We conclude that confirmation of BCR/ABL fusion gene or transcript can be very important in the diagnosis of CML even in the apparent presence of a Ph chromosome.

Further development and validation of a high-throughput targeted *BRCA1/BRCA2* mutation screening panel for women of African ancestry. *L. Gayol, M.E. Ahearn, T. Donenberg, B. Morel, J.F. Arena, L. Baumbach.* Univ Miami Sch Medicine, Miami, FL.

Recent evidence from our laboratory (Baumbach *et al.* AJHG 2003 73:#364), as well as others, suggest that certain *BRCA1/BRCA2* mutations and variants appear to be either at increased frequency or specific to women of African ancestry (AA) at-risk for breast cancer. Results agree that deleterious mutations in *BRCA1* or *BRCA2* are less frequent in these patients, suggesting that additional variants should be evaluated for their potential disease role. These observations have led to development of a screening panel for selected *BRCA1* and *BRCA2* mutations/variants in AA women. This panel is designed to detect 36 *BRCA1* mutations and unclassified variants, and 23 *BRCA2* mutations and unclassified variants, either previously detected in our laboratory, or found in a thorough review of all published English literature. The screening strategy is based on a combination of DNA sequencing, SSCP, and real-time PCR. Site-directed mutagenesis has been used to synthesize mutation-positive controls (as needed). Currently, the mutation-screening panel is comprised of 5 DNA sequencing reactions for *BRCA1*/ 4 DNA sequencing reactions for *BRCA2*, which together detect 86% of the mutations and variants, the remainder being detected by real-time PCR and/or SSCP. We have analyzed 40 patients using this panel, with another 70 to be completed by October. Two truncating mutations were detected in the 40 patients analyzed: *BRCA1* 943ins10 (founder mutation) and a rare *BRCA2* mutation (6828 delTT). Several rare missense mutations in *BRCA1* and *BRCA2* were also detected in one patient each, as well as a number of other recurrent missense mutations and unclassified variants in this cohort. We will present our cumulative study results in greater than 100 AA breast cancer patients. Eventual application of this panel to a large population-based study should contribute significant new information regarding frequencies and conferred risks of selected *BRCA1/BRCA2* mutations and variants. *The mutation screening panel has been granted preliminary patent protection through the University of Miami Office of Technology Transfer..*

Genetic testing for large germline deletions should be a clinical routine: *SDHD* and *SDHB* deletions as novel etiologies of heritable pheochromocytoma/paraganglioma syndrome (PC-PGL) as a model. *S.R. McWhinney*^{2,3}, *R. Pilarski*^{1, 2, 4}, *S. Forrester*⁵, *M. Schneider*⁵, *M.M. Sarquis*⁶, *E.P. Dias*⁷, *C. Eng*^{1, 2, 3, 4}. 1) Clinical Cancer Genetics Program; 2) Human Cancer Genetics Program; 3) Molecular Genetics; 4) Division of Human Genetics, Internal Medicine, Ohio State University, Columbus; 5) Pediatrics, S Illinois Univ Sch Med, Springfield; 6) Medicine, Federal University of Minas Gerais, Brazil; 7) Department of Endocrinology, Hospital Felcio Rocho Belo Horizonte, Federal University of Minas Gerais, Brazil.

Pheochromocytomas (PC) are catecholamine-secreting tumors that are primarily located in the adrenal medulla. Over 30% of PCs are hereditary. These neuroendocrine tumors are major components of 3 inherited cancer syndromes: multiple endocrine neoplasia type 2 (MEN 2), von Hippel-Lindau disease (VHL) and PC-PGL. Germline mutations in *RET*, *VHL*, and *SDHB*, *SDHC*, and *SDHD* are associated with MEN 2, VHL, and PC-PGL, respectively. The majority (>70%) of hereditary extra-adrenal PCs (secreting paragangliomas (PGL)) are accounted for by germline intragenic mutations in *SDHB*, *SDHC*, or *SDHD*. Therefore, a subset of hereditary PGL is not accounted for. Here we report 2 unrelated hereditary PGL families, one with a germline whole-gene deletion of *SDHD* (Fam4194), the other a partial deletion of *SDHB* (BRZ01). Although they were initially designated mutation negative for all the PC-associated genes after PCR-based analysis, we suspected that a large deletion or rearrangement might be present. Genotyping around the PC-associated genes indicated that Fam4194 and BRZ01 were consistent with linkage to *SDHD* and *SDHB*, respectively. Using fine structure genotyping and semi-quantitative duplex PCR analysis, we found that the deletion in both families segregated with the affected individuals. In addition, quantitative RT-PCR demonstrated a 4-fold decreased transcript expression in the proband compared to her unaffected mother, confirming the veracity of *SDHD* deletion in Fam4194. We were unable to obtain RNA from BRZ01. Therefore, including *SDHB* and *SDHD* deletion analysis could increase gene testing sensitivity for PGL patients, which would aid in genetic counseling and management of the patient and family.

Molecular Epidemiology of BRCA1 and BRCA2 Mutations in French Canadian Breast/Ovarian Families. *J. Simard¹, M. Dumont¹, A-M. Moisan¹, F. Durocher¹, R. Laframboise¹, M. Plante¹, J. Chiquette², B. Lesprance³, R. Pichette³, J. Lépine⁴, P. Bessette⁵, P. Voyer⁶, P. Bridge⁷, D. Goldgar⁸, INHERIT BRCA^s¹.* 1) CHUQ and Laval University, Quebec; 2) CHA, Hopital St-Sacrement, Quebec; 3) Hopital du Sacré-Coeur de Montréal; 4) Centre Hospitalier Régional de Rimouski; 5) Centre Universitaire de Santé de l'Estrie; 6) Carrefour de Santé de Jonquiere; 7) Alberta Childrens Hospital, Calgary; 8) International Agency for Research on Cancer, France.

Our study was designed to estimate the prevalence of deleterious mutations in BRCA1 and BRCA2 in French Canadian breast/ovarian cancer high risk families. To date, close to a thousand individuals from 251 families have been tested for at least 24 BRCA1 and BRCA2 deleterious mutations detected in the French Canadian founder population. Fifty-three possess a family history in both maternal and paternal sides, whereas in 61 families none of the individuals tested were affected. Our analysis led to the detection of the founder mutation R1443X (BRCA1) allele in 18 families, the 8765delAG (BRCA2) allele in 28 families. Eight additional truncating mutations were found in BRCA1 (n=5) and BRCA2 (n=3) by target sequencing. The complete sequence of all exons and splicing sites of BRCA1 and BRCA2 was done in 179 affected individuals or obligate carriers from 163 families, leading to the detection of 8 novel frameshift mutations and 25 sequence variants, in which 6 have been recently reclassified as polymorphisms. Based on our current extensive Southern blot and/or multiplex ligation probe amplification analyses, there is no evidence supporting the existence of any deleterious BRCA1/2 recurrent genomic rearrangement in the French Canadian population. The proportion of BRCA1/2-positive families among those showing within 1st or 2nd degree relatives of the index case an history of 3 (n=74), 4 or 5 (n=107) and 6 (n=53) of breast cancer cases, was 13.5%, 16% and 53%, whereas a mutation was found in 47% and 53% of families with 1(n=72) or 2 (n=30) ovarian cancer case(s). Note that a deleterious BRCA1/2 mutation was found in 58% of families with at least one (n=19) male breast cancer case.

Comparison of mRNA expression patterns in lymphoblasts from *BRCA1* mutation carriers and their wild-type relatives. *D.L. Stredrick*¹, *M.H. Greene*², *J.P. Struewing*^{1,2}. 1) Laboratory of Population Genetics, and; 2) Clinical Genetics Branch, National Cancer Institute, Bethesda, MD.

BRCA1 mutations are labor-intensive and costly to identify, but they are present in 3-5% of newly-diagnosed patients, and confer a very high risk of breast and ovarian cancer. mRNA expression studies have shown small, yet distinguishable differences between breast and ovarian cancers occurring in *BRCA1* mutation carriers versus non-carriers, and in one study of breast fibroblasts from 9 mutation carriers and 5 non-carriers after exposure to ionizing radiation. The ability to demonstrate differences between mutation carriers and non-carriers using expression analysis may provide a more efficient method for identifying carriers, and possibly give a more in-depth understanding of the normal function of *BRCA1*. We used Affymetrix U133A/B chips to compare mRNA expression in EBV lymphoblasts from 21 *BRCA1* mutation carriers and 21 of their wild-type relatives. Analysis was performed using BRB-ArrayTools, developed by the Biometric Research Branch of the NCI. Because large expression differences were not expected in the heterozygous carriers, *a priori* lists of candidate genes from the literature were used to guide the interpretation of our results. Two probe sets targeting the *BRCA1* gene revealed a significant, 20-25% lower expression among mutation carriers. After normalization using robust microarray normalization ("rma", part of Bioconductor), only 73 of 44,000 (0.16%) probe sets targeting other genes varied by 1.4-fold or more between the two groups, while scaling using MAS5 (target 500) revealed 3,236 (7.2%) probe sets that varied by this amount. Genes differentially expressed between the carriers and non-carriers were more likely to be on the *a priori* lists only when using significance analysis of microarrays (SAM) to compare the classes. Class prediction using leave-one-out cross-validation correctly classified 75% or fewer of the samples. Validation of the distinguishing genes will be performed using quantitative RT-PCR. More detailed analyses of these data are underway in an effort to determine the potential utility of this strategy.

Molecular genetic testing for hereditary leiomyomatosis and renal cell carcinoma in the clinical laboratory. *H. Sun¹, E. Fan¹, D. Melvin¹, R. Colliton¹, K. Hurley², L. Middleton², G. Glenn³, M-H. Wei^{3,4}, L. Schmidt⁴, B. Zbar⁵, J. Toro³, W.M. Linehan², C.A. Stolle¹.* 1) Path and Laboratory Medicine, Children's Hosp Philadelphia, Philadelphia, PA; 2) Urologic Oncology Branch, CCR, NCI, Bethesda, MD; 3) Genetic Epidemiology Branch, DCEG, NCI, Rockville, MD; 4) BRP, SAIC Frederick, Inc., NCI-Frederick, Frederick, MD; 5) Laboratory of Immunobiology, CCR, NCI-Frederick, Frederick, MD.

Hereditary leiomyomatosis and renal cell carcinoma (HLRCC) is an autosomal dominant condition in which affected individuals develop benign smooth muscle tumors of the skin; affected females frequently develop leiomyomata of the uterus (fibroids). Predisposition to type II papillary RCC has been observed in some families as an aggressive form of kidney cancer. Although several groups have recently identified mutations in the fumarate hydratase (FH) gene in patients with HLRCC, clinical molecular genetic testing has not been previously available. We have validated a molecular diagnostic test for HLRCC based on PCR amplification of 10 coding exons, conformation sensitive gel electrophoresis, and DNA sequence analysis of shifted exons. To date we have analyzed 51 patients for point mutations in the FH gene, including 20 probands and 31 at risk family members. Point mutations were detected in 18 of 20 probands for a mutation detection rate of 90%. Among the mutations identified, 7 were novel including 4 missense (R117G, R190C, A342D, and S376P), 1 nonsense (S102X), 1 frameshift (ins A nt 110-111; FS38->X51), and 1 splice site (138 +1 G>C) mutation. Novel missense mutations were considered potentially disease causing since they resulted in non-conservative amino acid substitutions, altered evolutionarily conserved amino acid residues, and were not found in over 100 normal alleles. Two patients did not exhibit a detectable point mutation. Preliminary Southern blot analysis failed to detect a partial or complete deletion of the FH gene. Since loss of expression of the FH gene may result in HLRCC, additional methods, such as quantitative PCR analysis of mRNA levels or PR-PCR analysis of mRNA splicing products, may be helpful for detecting mutations in these patients.

CHK2 1100delC Mutation and Inherited Breast Cancer Susceptibility in Alberta, Canada. *B. H Sun¹, S. Zhang², L. Bai¹, K. McLachlan¹, M. Lilley³, J. Liu¹, M. Somerville⁴, D. Gilchrist⁴, R. Hughes¹.* 1) Oncology, Univ. Calgary, AB; 2) Public Health Sci., Univ. Toronto, ON; 3) Population Health Info., Edmonton, AB; 4) Genetics, Univ. Alberta, Edmonton, AB.

Introduction: Mutations in BRCA1 and BRCA2 genes confer a high risk for developing breast and ovarian cancer, but account for only 25% of hereditary breast ovarian cancer (HBOC). The discovery of germline CHK2 gene mutations in breast cancer patients from HBOC families suggests that this may be the third BRCA gene to be identified. CHK2 gene encodes a cell-cycle checkpoint kinase that is a key mediator of cellular response to DNA damage. A recent CHK2 Breast Cancer Case-Control Consortium has demonstrated a frameshift mutation of CHK2 gene, 1100delC, which abrogates checkpoint kinase activity, is associated with an increased frequency of breast cancer. Analysis of this variant in Alberta Families with hereditary breast cancer is important to determine if it is predictive of cancer risk in our population. **Subjects:** Cases-135 females diagnosed with breast cancer from HBOC families without BRCA gene mutations. Controls- 279 healthy, age-matched females who underwent routine blood testing in the Calgary region. **Methods:** Long-range PCR was used to amplify CHK2 exons 10 to 14 that contains regions with high homology to CHK2 pseudogenes. Second set of PCR primers used that contain one base substitution used to generate a restriction enzyme site for ScaI within the wild-type allele of CHK2 exon 10. CHK2 1100delC mutation was detected by agarose gel analysis of ScaI digested PCR products. **Results:** The age range in case group is 30-87 years with an average of 57.7 11.9 years. The age range in control group is 30-88 years with an average of 57.9 12.5 years. The frequency of CHK2 1100delC was 1/135 (0.74%) in cases with a family history of breast cancer, compared to a frequency of 1/279 (0.36%) in healthy controls (odds ratio 2.07; 95% CI 0.13-33.35; $p > 0.05$). **Conclusions:** CHK2 1100delC is not a common cause of familial breast cancer. No significant difference between the case and control groups in our population was identified.

Significant contribution of germline *BRCA2* rearrangements in male breast cancer families. *M. Tosi*¹, *I. Tournier*¹, *B. Bressac-de Paillerets*², *H. Sobol*³, *D. Stoppa-Lyonnet*⁴, *R. Lidereau*⁵, *M. Barrois*², *S. Mazoyer*⁶, *F. Coulet*⁷, *A. Hardouin*⁸, *A. Chompret*², *P. Chappuis*⁹, *T. Frébourg*¹. 1) Faculté de Médecine & Pharmacie, Inserm U614, Rouen, France; 2) Service de Génétique, Institut Gustave Roussy, Villejuif, France; 3) Inserm EPI 9939 Institut Paoli-Calmettes, Marseille; 4) Service de Génétique Oncologique, Institut Curie, Paris; 5) Centre René Huguenin, Inserm E0017, Saint-Cloud, France; 6) Laboratoire de Génétique, Unité Mixte de Recherche 5641 CNRS, Lyon, France; 7) Génétique Moléculaire, Hôpital Tenon, Paris, France; 8) Centre François Baclesse, Caen, France; 9) Service d'Oncologie, Hôpital Universitaire de Genève, Suisse.

While screening for large deletions or duplications of the *BRCA1* gene is becoming a routine component of the molecular diagnosis of familial breast cancer, little is known about the occurrence of such rearrangements in *BRCA2*. Because of the high frequency of *BRCA2* mutations in breast cancer families with at least one case of male breast cancer, we selected a cohort of 39 such families, tested negative for mutations in the coding regions of *BRCA1* and *BRCA2*, and developed an assay for *BRCA2* rearrangements, based on quantitative multiplex PCR of short fluorescent fragments (QMPSF). We found three rearrangements: a deletion of exons 12 and 13, a duplication of exons 1 and 2 and a complete deletion of *BRCA2*. We determined the boundaries of the deletion of exons 12 and 13, showing that it resulted from unequal recombination between *Alu* sequences. We mapped the complete *BRCA2* deletion, which extends over at least 298 kilobases and demonstrated that it does not affect *APRIN/AS3*, previously characterized as a tumor suppressor gene, but it comprises several loci corresponding to proven or putative transcripts of unknown functional significance. We found no *BRCA2* rearrangement in a cohort of 91 families with breast and ovarian cancer, in which we had previously found 5 distinct germline rearrangements of *BRCA1*. These data suggest that screening for *BRCA2* rearrangements should be performed in male breast cancer families tested negative for *BRCA1/2* mutations and possibly also in other families with multiple cases of breast cancer.

A new family with both juvenile polyposis and hereditary haemorrhagic telangiectasia caused by a mutation in MADH4. *G. Viot*¹, *S. Epelboin*², *F. Soubrier*³, *A. Handra-Luca*⁴, *S. Olschwang*⁴. 1) Dept Genetics, Hosp Necker, Paris, France; 2) Gynecology, hosp Saint Vincent de Paul, Paris, France; 3) Unity of Molecular Genetic, Hosp Tenon, Paris, France; 4) Unity of Molecular Genetic, Hosp Saint Antoine, Paris, France.

In 1980, the association of juvenile polyposis (JP) and hereditary haemorrhagic telangiectasia (HHT) was reported for the first time. JP is caused by mutations in MADH4 (encoding SMAD4) and BMPR1A genes; HHT by mutations in ENG (endoglin) or ACVRL1 (ALK1). The genetic aetiology of the association of both disorders was unknown until recently when Gallione et al. reported mutations in MADH4 resulting in this combined syndrome. We report a new three generations family with 8 affected members. All of them met criteria of HHT (pulmonary arteriovenous malformation, cutaneous telangiectasia, epistaxis). Because one of the probands exhibited a colorectal cancer, endoscopy was performed for all members. Only those with HHT had hamartomatous polyps throughout the gastrointestinal tract. Subtotal colectomy was performed for 4 of them. No mutation in the ENG or ACVR1 genes was found. On the other hand, one mutation (G1571T, W524L) was identified in the 11th exon of the MADH4 gene, which has never been previously reported. This new report confirms that the genetic testing of the MADH4 gene is recommending in all patients presenting both HHT and JP.

Loss of Heterozygosity in Primary Breast Carcinomas and Metastatic Tumors of the Axillary Lymph Nodes. *R.E. Ellsworth¹, D.L. Ellsworth¹, B. Deyarmin¹, M. Sarachine¹, P. Brown¹, J. Hooke², C.D. Shriver²*. 1) Clinical Breast Care Project, Windber Research Inst, Windber, PA; 2) Clinical Breast Care Project Walter Reed Army Medical Center, Washington, DC.

Despite tremendous advances in early detection and treatment, breast cancer remains the second leading cause of cancer-related death in women. While a number of clinical parameters have been identified, axillary lymph node status remains the most important prognostic factor in predicting disease outcome. Many studies have examined chromosomal aberrations in primary tumors and correlated these findings with lymph node status and clinical outcome, but chromosomal changes particular to axillary lymph nodes have not been well studied. In this study, we used loss of heterozygosity (LOH) analysis to examine 26 of the most commonly deleted regions in breast cancer in both primary breast tumors and metastatic tumors in the axillary lymph nodes. DNA was isolated after laser-assisted microdissection of pure tumor cell populations to improve the accuracy of LOH detection. Of note, chromosomal regions 9p21 and 13q12.3 were lost in some primary tumors but never in the lymph node tumors, suggesting that genes within these regions may not be necessary for lymphogenic spread. In contrast, both primary and lymph node tumors retained regions 14q31, 7q31 and 11p15, regions known to harbor the MTA1 gene, which serves to promote metastasis, and the SPAM1 and MMP26 genes, both responsible for extracellular matrix degradation and possibly tumor dissemination. Finally, the primary tumors showed significantly more loss than did the lymph node tumors especially at chromosomal regions 8p22-21.3, and 22q12.3, suggesting that the detachment of cells with metastatic potential may be an early event in tumor progression, occurring before the accumulation of gross genetic alterations.

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High Resolution Array CGH Profiling of Small Cell Lung Cancer Cell Lines. *E.H.L. Lee, B.P. Coe, S. Lam, C. MacAulay, W. Lam.* Cancer Genetics and Dev. Biol., BC Cancer Agency, Vancouver, BC, Canada.

Small-cell lung cancer (SCLC) represents ~25% of all lung cancers and is rarely cured with present therapies. The median survival time is only ~12-16 months. There is a need to identify molecular targets for prognosis and therapy of the disease to increase survival rates. Array comparative genomic hybridization (aCGH) is currently the highest resolution method for detecting genomic alterations in human cancers. We have developed a CGH array consisting of 32,433 overlapping BAC clones covering the entire human genome. 15 SCLC cell lines were profiled using the SMRT array in order to identify and implicate genes contributing to the disease phenotype. To this end, DNA from the cell lines was isolated in sufficient quantities for SMRT array CGH profiling. In-house SeeGH software allowed identification of multiple recurrent gains and losses of genetic material, including previous reported alterations at the hTERT, ATM, and MYC loci. In addition, we were able to resolve chromosome breakpoints to within a single BAC clone and we detected novel micro-amplifications and micro-deletions as small as 200kb that would be undetectable with conventional methodologies. These novel genetic alterations will further our understanding of the biology of SCLC.

Genome-wide loss of heterozygosity analysis of *WT1*-wildtype and *WT1*-mutant Wilms tumors. *E.C. Ruteshouser*¹, *B.W. Hendrickson*^{1,2}, *S. Colella*^{1,2}, *R. Krahe*^{1,2}, *V. Huff*^d. 1) Department of Molecular Genetics, Section of Cancer Genetics, The University of Texas M. D. Anderson Cancer Center, Houston, TX; 2) Human Cancer Genetics Program, Department of Molecular Virology, Immunology, and Medical Genetics, Comprehensive Cancer Center, Ohio State University, Columbus, OH.

Wilms tumor (WT) is genetically heterogeneous, and the one known WT gene, *WT1* at 11p13, is altered in only 20% of WT. Previous loss of heterozygosity (LOH) analyses have revealed the existence of additional putative WT genes at 11p15, 16q, and 1p, but these analyses examined only one or a handful of chromosomes, or looked at LOH at only a few markers per chromosome. Because *WT1* is a transcriptional regulator with effects on the expression of a variety of target genes, we hypothesized that tumors carrying *WT1* mutations would display lower levels of LOH than *WT1*-wildtype tumors since mutations in *WT1* would result in the dysregulation of expression of genes which would, in *WT1*-wildtype tumors, otherwise be dysregulated via genetic alterations including LOH. Accordingly, we conducted a genome-wide scan for LOH in WT using 420 microsatellite markers spaced at an average of 10 cM throughout the genome and analyzed the data for two genetically defined subsets of WT: those with mutations in *WT1* and those with no detectable *WT1* alteration. Our findings indicate that the incidence of LOH throughout the genome is dramatically lower in our group of WT with *WT1* mutations. In *WT1*-wildtype tumors, we observed the expected LOH at 11p, 16q, and 1p, and in addition localized a previously unobserved region of LOH at 9q. Using additional 9q markers located within this region of interest, we sublocalized the region of 9q LOH to the 15.5 Mb between D9S283 and D9S1784, a region containing several potential tumor-suppressor genes. As a result, we have established for the first time that *WT1*-mutant and *WT1*-wildtype WT differ significantly in their patterns of LOH throughout the genome, suggesting that the genomic regions showing LOH in *WT1*-wildtype tumors harbor genes whose expression is regulated by the pleiotropic effects of *WT1*. Our results implicate 9q22.3 - q31.1 as a region containing such a gene.

Whole genome array CGH identifies micro-genetic alterations in cancer. *J.J. Davies, C. Garnis, E. Vucic, C. Baldwin, R.J. deLeeuw, S.K. Watson, C. MacAulay, W.L. Lam.* British Columbia Cancer Agency, Vancouver, BC, Canada.

Array-based comparative genomic hybridization (CGH) is a technique that is widely used to study genomic DNA copy number changes. By competitively hybridizing differentially labeled tumor and normal reference DNA to an array, one can determine a tumor's genomic integrity at specific marker loci. In the past, changes in genetic copy number were inferred for gaps between marker loci. Recently we have constructed a DNA array that covers the entire human genome with >32,000 overlapping genomic segments in the form of DNA derived from bacterial artificial chromosome (BAC) clones. The contiguous arrangement of clones allows for the complete assessment of an entire genome in a single experiment, thereby removing the assumption of continuity between marker loci. At this unprecedented resolution, we have identified minute genetic aberrations that have evaded characterization by conventional cytogenetic techniques. Fingerprint verified BAC clones were converted to amplified fragment pools (AFPs) via linker mediated PCR. Following re-verification by AFP sequencing, the AFPs were spotted onto aldehyde slides in triplicate. Differentially labeled sample and reference DNA were hybridized to the array followed by imaging and analysis. Software called SeeGH was created to visualize the substantial amount of data describing each genome. Using this comprehensive approach, we have detected minute aberrations in the genome that have not been previously reported. These alterations include micro-amplifications and deletions harboring known tumor suppressors and oncogenes, and novel candidate genes. Minute genetic alterations were discovered in lung, oral, breast, prostate and lymphoid cancers. Our findings demonstrate the need to move beyond conventional marker-based genome comparison, which rely on inference of continuity between interval markers; and that sub-megabase micro-amplifications and deletions are common events in cancer. This work is a component of a cancer genomics project, supported by funds from Genome Canada/Genome British Columbia.

FANCA sequence variations in high-risk non-BRCA1/2 French Canadian families. *S. Desjardins, M. Ouellet, Y. Labrie, G. Ouellette, J. Simard, INHERIT BRCA's, F. Durocher.* Cancer Genomics Laboratory, CHUL Research Ctr, CHUQ, Laval University, Quebec, PQ, Canada.

Sequence alterations in FANCA account for more than 60% of Fanconi Anemia cases, a rare recessive disease characterized by bone marrow failure, genomic instability and predisposition to cancer. FANCA is implicated in DNA repair as part of the FANC complex and has been shown to directly interact with BRCA1. A growing number of evidence links Fanconi Anemia with BRCA1 and BRCA2 genes, which are known to be involved in inherited susceptibility to breast and ovarian cancer. Since a minority of inherited breast cancers is attributable to BRCA1 and BRCA2 mutations, FANCA therefore becomes an attractive candidate for breast cancer susceptibility. We thus analyzed the FANCA gene in more than 40 high-risk French Canadian families in which no BRCA1 or BRCA2 mutations have been identified. A total of 13 sequence variants were found: 8 polymorphisms (3 silent and 5 missense changes) and 5 changes that could affect splicing. Three known missense changes (Thr266Ala, Ala412Val and Ser501Gly) and 2 possible alternative splicing events fall within the BRCA1- and BRG1-interacting region, which is a subunit of the SWI/SNF complex implicated in chromatin remodelling. The other 2 missense variants (Ser1088Phe and Thr1328Ala) and the other 3 possible alternative splicing events take place within the FANCC-interacting region, which is essential for the formation of the FANC complex and DNA repair. The Ser1088Phe variant has already been associated with Fanconi Anemia. The alternative mRNA forms have been analysed by specific probe hybridization and their presence has been investigated in 13 cell lines and 12 normal tissues. Multiplex ligation-dependent probe amplification (MLPA) was used to verify the integrity of the genomic DNA, as FANCA has also been shown to present a high rate of ALU-mediated genomic deletions. Sequence variants in FANCA could therefore be potential spoilers of the Fanconi-BRCA pathway and as a result, they could have an impact in non BRCA1/2 breast cancer families.

Characterization of sequence variants in high-risk non-BRCA1/2 French Canadian families. *Y. Labrie¹, F. Durocher¹, P. Soucy¹, D. Labuda², J. Simard¹, INHERIT BRCA's.* 1) Cancer Genomics Laboratory, CHUL Research Ctr, CHUQ, Laval University, and; 2) Centre de cancérologie Charles Bruneau, Ste-Justine Hospital, Québec, Canada.

Ataxia telangiectasia-mutated and Rad3-related (ATR) is a member of the PI3K-related family which plays, along with ATM, a central role in cell-cycle regulation, by transmitting DNA damage signals to downstream effectors of cell-cycle progression. Furthermore, ATR has been shown to phosphorylate BRCA1. These findings therefore render ATR an attractive candidate susceptibility gene for breast cancer, and thus the goal of this study was to evaluate the possible involvement of ATR sequence variants on breast cancer susceptibility. The complete sequence of the 47 exons and flanking intronic sequences of the ATR gene was therefore analyzed in 55 affected individuals from 55 high-risk French Canadian families in which no mutation in either BRCA1 or BRCA2 has been identified. No putative deleterious mutation leading to a premature termination of the protein was identified in the coding region. However, 42 sequence variants were identified, among which 16 are coding variants, 14 being novel changes. All coding variants were also analyzed in a cohort of 100 healthy unrelated individuals of French Canadian origin. In the affected cohort, 5 variants, all with a rare allele frequency of less than 5%, seem to deviate significantly from Hardy-Weinberg expectations, with an excess of rare homozygotes. Moreover, among the 16 coding variants, 6 are non-conservative changes and 2 are located in the FAT and kinase domains involved in the ATR protein activity. Protein sequence alignments with 14 other species show that 2 changes are conserved in at least 2 other species. Among the 10 silent coding polymorphisms identified, five display a frequency greater than 10%. Additional studies in international cohorts are currently underway to further evaluate the implication of these sequence variants in cancer susceptibility.

DNA damage induced by curcumin in the presence of copper in an in vitro murine model evaluated by comet assay. *P. Urbina-Cano*¹, *L. Bobadilla-Morales*¹, *M. Ramírez-Herrera*², *A. Corona-Rivera*¹. 1) Laboratorio de Genética Humana, Departamento de Fisiología, CUCS, Universidad de Guadalajara. México; 2) Laboratorio de Neurofisiología, Departamento de Fisiología, CUCS, Universidad de Guadalajara. México.

Curcumin had shown a wide range of pharmacological properties including anti-inflammatory, anti-tumor and antioxidant effects. More specifically, because of their antioxidant properties, inhibit oxidative damage to cellular DNA, and thereby, prevent mutagenesis and tumorigenesis. Most of its biological effects are due to its antioxidant and radical scavenging properties. Some antioxidants are capable of causing strand breakage in DNA in the presence of transition metal ions as copper. Such activity could be physiologically relevant in view of elevated copper ion concentrations in various diseased conditions. The goal of our study was to investigate the effect of curcumin 50M in the presence of increasing concentrations of Cu (II) to cellular DNA with the alkaline comet assay. Murine lymphocytes were incubated with curcumin 50M and different concentrations of Cu (II) (10M, 100M and 200M) for 1h at 37C in a CO2 incubator. The cells were washed and resuspended in PBS. To detect cellular DNA damage alkaline micro-gel electrophoresis (comet assay) was performed essentially as described by Singh and coworkers. To evaluate the DNA damage, comets were Propidium Iodide stained and tail length measured. Tail length average show that curcumin in the presence of Cu (II) 100 M and 200 M but not 10 M induced DNA damage to murine lymphocytes. Curcumin 50 M in the presence of Cu (II) 100 M caused the highest DNA damage. The curcumin 50 M was also able to directly induce DNA damage to murine lymphocytes. Our data suggest that curcumin in the presence of copper increases murine lymphocytes DNA damage detected by comet assay.

Genomic Deletions Involving Homologous Alu Sequences May Be More Common in MSH2 than in MLH1. *L. Li^{1, 3}, S. Mcvety³, R. Younan^{2, 4}, I. Thiffault^{1, 3, 4}, D. DuSart⁵, P. Hutter⁶, F.B. Hogervorst⁷, G. Chong^{1, 3, 4}, W.D. Foulkes^{1, 3, 4}.* 1) Program in Cancer Genetics; 2) Dept. of Surgery; 3) Dept. of Human Genetics; McGill University, Montreal, QC, Canada; 4) Dept. of Diagnostic Medicine, SMBD-Jewish General Hospital, Montreal, QC, Canada; 5) Murdoch Childrens Research Institute, Royal Childrens Hospital, Victoria, Australia; 6) Institut Central des Hpitaux Valaisans, Sion, Switzerland; 7) Family Cancer Clinic, Netherlands Cancer Institute, Amsterdam, Netherlands.

Genomic rearrangement is an important component of the mutation spectrum of mismatch repair genes MSH2 and MLH1, and has been implicated in several reported founder mutations in hereditary non-polyposis colorectal cancer (HNPCC). In this study, we identified the DNA breakpoints in the MLH1 and MSH2 genes in 10 patients, who tested positive for large genomic deletions by Southern hybridization, protein truncation test (PTT) or multiplex ligation-dependent probe amplification (MLPA). Long-range polymerase chain reaction and sequencing revealed distinct characteristics of the sequences mediating the deletions in the two genes. Among the 5 cases of MSH2 deletions, 4 result from intragenic homologous recombination mediated by repetitive Alu sequences. A notable feature of these Alu mediated deletions is that the core sequences immediately adjacent to the breakpoints from both ends share high identity. However, in one case of MSH2 deletion, the upstream and downstream breakpoints are located in Alu and L1 elements, respectively, and the two sequences do not share significant homology. The molecular mechanism underlying the 5 cases of genomic deletions in MLH1 appear to be more complex. The DNA breakpoints are located in Alu, L elements as well as non-repetitive sequences. No specific sequence pattern has been observed. Our study suggests that the intragenic deletions in MLH1 result from more complex recombination events compared to the deletions in MSH2. Dissecting the molecular basis of large genomic deletion in MLH1 and MSH2 will ultimately contribute to improving the diagnostic approach for this type of mutation and moreover, may provide mechanistic insights into their origin.

Insulin-like growth factor-1 (*IGF1*) genotype predicts breast volume after pregnancy and hormonal contraception and is associated with circulating IGF-1 levels: implications for risk of early-onset breast cancer in young women from hereditary breast cancer families. *H. Jernstrom¹, T. Sandberg¹, A. Borg¹, H. Olsson².* 1)

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BRCA1/2 mutations predispose to early-onset breast cancer, especially after oral contraceptive (OC) use and pregnancy. However, the majority of breast cancers might be due to more prevalent low penetrance genes, which may also modify the risk in BRCA mutation carriers. The absence of the *IGF1* 19-repeat allele has been associated with high insulin-like growth factor-1 (IGF-1) levels during OC use. High IGF-1 levels are linked to early-onset breast cancer and larger breast volumes in the general population. The goal of this study was to elucidate the relationships between *IGF1* genotype, early-onset breast cancer, breast volume, circulating IGF-1 levels and OC use in a prospective cohort of 258 healthy women 40 years-old from high-risk breast cancer families. All women completed a questionnaire including information on reproductive factors and OC use. We measured height, weight, breast volumes and plasma IGF-1 levels. IGF-1 levels were similar among parous and nulliparous women not using OCs. Thirteen percent had no *IGF1* 19-repeat allele. There was an interaction between *IGF1* genotype and OC use on IGF-1 levels ($p=0.026$) in nulliparous women and another interaction between *IGF1* genotype and parity on breast volume ($p = 0.01$). Absence of the 19-repeat allele was associated with high IGF-1 levels in nulliparous OC users and with larger breast volumes in parous women and OC users. Incident breast cancers were also more common in women without the 19-repeat allele (Log-rank $p = 0.002$). Our results suggest that lack of the *IGF1* 19-repeat allele modifies IGF-1 levels, breast volume and possibly early-onset breast cancer risk after hormone exposure in young high-risk women.

Overexpression of Her2 and Ki67 is associated with breast tumor aggressiveness. *N. Tian*¹, *S. Yan*², *Q. Wang*³, *W. Qiao*⁴, *S.W. Fu*⁵. 1) Surgery, Fugu Hospital, Fugu, Shaanxi, China; 2) Dept. of OB and GYN, Affiliated Teaching Hospital, Tibet Nationalities Institute, Xianyang, Shaanxi, China; 3) Dept. of Surgery, Shengmu Hospital, Shengmu, Shaanxi, China; 4) Dept. of Medicine, The 2nd Affiliated Hospital, Xi'an Jiaotong University, Xi'an, Shaanxi, China; 5) Dept. Biochemistry & Molecular Biology, George Washington University, Washington, DC.

The aggressiveness and metastasis of breast cancer is the most insidious and life-threatening risk for breast cancer patients. Clearly, the aberrant expression of genes controlling cell growth and proliferation in tumor cells plays an important role. This study was to explore the potential effects of the expression of Her2 and Ki67 genes in breast cancer. Formalin-fixed, paraffin-embedded human breast tumor tissues were used, and immunohistochemical staining were carried out using ABC method to determine the expression of HER-2, Ki67, in primary breast carcinoma patients. The X^2 test was used to compare immunohistochemical results for Her2 and Ki67 with clinico-pathologic characteristics, such as patient age, tumor size, lymph node status, histological grade, and ER and PR status. Of the 87 primary invasive ductal carcinomas, 24.6% and 61.4% were positive for HER2 overexpression and Ki67 expression, respectively. HER2 overexpression was significantly correlated with tumor size ($p = 0.002$), number of positive lymph nodes ($p = 0.004$), and histological grade ($p < 0.0001$). There was an inverse association between HER2 overexpression, and ER expression ($p < 0.002$) as well as PR expression ($p < 0.003$). Ki67 expression was significantly correlated with histological grade ($p = 0.003$), whereas no association was found between Ki67 expression and tumor size, number of positive lymph nodes, ER or PR expression. Further analysis indicated that there was a significant association between expression of Her2 and that of Ki67 ($p < 0.001$), and Her2 overexpression and Ki67 protein accumulation are associated with aggressive breast cancer.

Using Brother Pairs Discordant for Prostate Cancer to Identify Prostate Cancer Susceptibility Genes. K.A.

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Family history is a well-recognized risk factor for prostate cancer. To identify cancer susceptibility genes, we have established the University of Michigan Prostate Cancer Genetics Project (PCGP), a family-based study of hereditary prostate cancer. Within a subset of PCGP families, we have identified sets of brother pairs discordant for prostate cancer affection status in order to study susceptibility genes with moderate penetrance. We have recently genotyped 717 brothers with and without prostate cancer from PCGP families using one common single nucleotide polymorphism (SNP) from each of five prostate cancer candidate genes: CYP17 (10q24.3), CYP19 (15q21.1), BRCA1 (17q21), CYP11A (15q23-24), and LHB (19q13.32). Genotyping was performed using the ABI PRISM 7900HT Sequence Detection System. There were 377 men with prostate cancer and 340 unaffected brothers from 266 families included in this analysis. Families were primarily Caucasian, although 12 African American and 2 Asian families were also included. To date, we have detected significant associations between prostate cancer and SNPs in BRCA1 and CYP17 using the Family Based Association Test (FBAT) package. Both SNPs display significant association with prostate cancer in an affecteds-only analysis ($p = 0.020$ and $p = 0.010$). The SNPs in BRCA1 ($p = 0.019$) and CYP17 ($p = 0.003$) are also significant when unaffected brothers are included. Although preliminary, the findings with regard to BRCA1 are consistent with our evidence of linkage to 17q21 in a set of overlapping families from the U of M PCGP. Given the latter linkage result, our family-based association study design which protects against spurious associations arising from population admixture, and other independent reports of association and/or linkage, both genes merit further exploration and testing in the current sample and a future replication sample. This work may lead to the identification of a set of SNPs that could be used for prostate cancer risk stratification in men with a family history of the disease.

Characterization of variation across the human TP53 gene. *L. Burdett*¹, *S. Presswala*¹, *V. Llaca*¹, *M. Yeager*¹, *D. Gerhardt*², *R. Strausberg*³, *V. Kristensen*⁴, *C. Perou*⁵, *A. Borrensens-Dale*⁴, *S. Chanock*⁶. 1) Core Genotyping Facility/GAI, SAIC-Frederick/NCI, Gaithersburg MD; 2) OCG/NCI/NIH Bethesda MD; 3) TIGR Gaithersburg MD; 4) Dept of Genetics, Norwegian Radium Hospital, Oslo Norway; 5) Lineberger Cancer Center, UNC, Chapel Hill NC; 6) POB/NCI/NIH, Bethesda MD.

Characterization of variation across the TP53 gene was performed to set a baseline for studies of the gene in breast cancer patients. Resequencing of 7000bp of TP53 (including all exons, 2500bp 5' and 1000bp 3') in a panel of 102 individuals of 4 self-described ethnic groups (N=24 African/African-Americans, N=31 Caucasians, N=23 Hispanics, and N=24 Pacific Rim) and N=94 Norwegian woman revealed several novel single nucleotide polymorphisms. The variant encoding the arginine of the well-studied R72P polymorphism was observed at allele frequency of 0.54 across the populations with a range of 0.29 for African-Americans to 0.73 for Caucasians. Fifteen SNPs were observed at a frequency greater than 5% and were used to characterize haplotypes. Phase was estimated and then confirmed by pedigree analysis of 16 CEPH families. There were 3 haplotypes that explain 80% of haplotypic variation in the Caucasians, of which two are the major haplotypes in the Hispanic group studied. The Pacific Rim group contains 2 major haplotypes, only one of which overlaps with the Caucasians. As may be expected, the African/ African-American group contains more haplotypes overall, with lower frequency than in the other groups. Two htSNPs, rs1614984 and rs1042522, describe 95% of the haplotypes for Caucasians as well as the Hispanic. The Pacific Rim group requires one additional htSNP and 4 are required for the African/African American group with only one htSNP, rs1614984, overlapping with the htSNPs of the other groups. Further sequence analysis of the genes neighboring TP53, show an LD block that extends 5' through two neighboring genes, FLJ10385 and EFNB3, and in the 3' direction into ATP1B2.

ATM haplotypes and breast cancer in Jewish high risk women. *E. Friedman*^{1,3}, *M. Koren*¹, *G. Kimmel*², *S. Eisenberg-Barzilai*¹, *R. Shamir*². 1) Oncogenetics Unit, Inst Gen, Chaim Sheba Medical Ctr, Tel Hashomer, Israel; 2) Department of Mathematics, Tel-Aviv University, Tel-Aviv, Israel; 3) Sackler school of medicine, Tel-Aviv University Tel-Aviv Israel.

The contribution of ATM gene mutations to breast cancer risk in the average- and high-risk populations is unsettled. To shed light on this issue, we constructed ATM haplotypes using 8 intragenic SNPs spanning the 142Kb genomic region of the ATM gene in unaffected, ethnically diverse non-Ashkenazi Jewish individuals (controls - n=111), using a novel expectation-maximization algorithm (GEBRIL). Of the 15 haplotypes noted, 4 were encountered in frequencies of 5% or more, and combined accounting for more than .% of all haplotypes, with extensive LD among all ethnically diverse groups. Subsequently, ATM haplotyping of high risk Jewish individuals of Ashkenazi [n=107, all breast cancer patients all carriers of BRCA1 (n=33) or BRCA2 (n=64) mutations] and non Ashkenazi (n=227; 115 breast cancer, 112 unaffected of whom none was a BRCA1/2 mutation carrier) origin was carried out. The results showed that one haplotype, coined haplotype 2 SPECIFY!!! was significantly more common in high risk, affected and unaffected, women (20%) than controls (5%) (p=0.0005), and was noted in 31% of high risk cancer cases, (p=0.0005 Vs controls and p=0.03 vs high risk unaffected). Analysis of ATM haplotype distribution by age at diagnosis (<40; 41-49; >50+ years) in BRCA1 (n=33) and BRCA2 (n=64) affected mutation carriers failed to demonstrate specific haplotype clustering by age at diagnosis in either group. This preliminary study suggests that a specific ATM haplotype is associated with elevated breast cancer risk in Jewish non-Ashkenazim and that ATM probably does not function as an age at diagnosis modifier of BRCA1/2 Jewish Ashkenazi mutation carriers.

RAD51 haplotypes and breast cancer risk in Jewish women. *I. Gal¹, R. Gershoni-Baruch², G. Kimmel³, M.Z. Papa⁴, E. Dagan², R. Shamir³, E. Friedman¹.* 1) The Susanne Levy Gertner Oncogenetics Unit, Institute of Genetics, Tel-Hashomer, Israel; 2) The Genetics Institute, Rambam Medical Center, Haifa, Israel; 3) Faculty of Exact Sciences, School of Computer Science, Tel-Aviv University, Tel-Aviv, Israel; 4) The Department of Oncological Surgery, Sheba Medical Center, Tel-Hashomer, Israel.

While the precise genes involved in determining breast cancer risk, are mostly unknown, one strong candidate is the RAD51 gene. To further evaluate RAD51 putative role in breast cancer susceptibility, RAD51 haplotypes were determined in Jewish non-Ashkenazi high risk women and among Jewish BRCA1 BRCA2 mutation carriers. The study population encompassed women at high risk for breast/ovarian cancer and an ethnically matched control group. Four SNP's spanning the entire RAD51 genomic region was scored using the Pyrosequencing technology, and the resulting haplotypes were constructed using the GERBIL algorithm. A total of 550 women were genotyped: 144 BRCA1 mutation carriers (71 with breast/ovarian cancer); 85 BRCA2 mutation carriers (26 with breast/ovarian cancer); 191 non-Ashkenazi high risk women (127 with breast/ovarian cancer); 130 unaffected controls. Control group genotyping showed that all SNP's maintained the Hardy Weinberg Equilibrium, and did not show any ethnic differences. Using GEBRIL three frequent haplotypes could be constructed: haplotype 1-CCTG (47.6%), haplotype 2-TGGG (39%) and haplotype 3-TGTA (13.4%). Haplotype 3 was present in 7.3% (19/260 haplotypes) of controls (n=130) and in 15.5% (131/840 haplotypes) of all cases (n=420) (p= 0.0021). Compared with controls, haplotype 3 was significantly more prevalent among breast cancer cases (regardless of BRCA mutational status) 16% (72/448 haplotypes) (n=224, P=0.0023), among non carrier breast cancer patients 17% (43/252 haplotypes)(n=126, p=0.0024), and among BRCA1/2 mutation carriers regardless of disease status 15% of 97 affected mutation carriers (p=0.026), and 16% among 132 asymptomatic carriers (p=0.006)]. It seems that a specific RAD51 haplotype is associated with an increased breast cancer risk in Jewish high risk women and in BRCA1/2 mutation carriers.

Support for an Ashkenazi Jewish origin for the *BRCA1*185delAG* mutation observed in the non-Jewish San Luis Valley population. *I. Makryianni*¹, *N. Hamel*^{1,2}, *W. Foulkes*^{1,2}, *S. Graw*³. 1) Department of Human Genetics, McGill University, Montreal, Canada; 2) Department of Medicine, McGill University Health Centre, Montreal, Canada; 3) Eleanor Roosevelt Institute, University of Denver, Denver, Colorado.

Numerous *BRCA1* mutations predispose to breast and ovarian cancer. *BRCA1*185delAG* is the most frequent *BRCA1* mutation in the Ashkenazi Jewish (AJ) population. In a Chilean family with no known Jewish ancestry, Ah Mew et al. (Clin Genet 2002; 62: 151-56) found that *BRCA1*185delAG* occurred on a haplotype identical to that observed in the AJ population. This mutation has also been identified in the Hispanic population of the San Luis Valley of southern Colorado and northern New Mexico. In this study we set out to determine whether *BRCA1*185delAG* found in this region has occurred on the previously recognised AJ haplotype. The San Luis Valley was first settled by the de Onate expedition in 1598 and some of these settlers may have had Jewish ancestors. However, individuals involved in this study did not report any Jewish ancestry. Individuals diagnosed with either breast cancer or ovarian cancer met the ASCO genetic testing for cancer predisposition inclusion criteria prior to genetic testing. Myriad Genetics identified the *BRCA1*185delAG* mutations. We genotyped these individuals for 7 markers located within or near *BRCA1*. Unrelated AJ *185delAG* carriers were used as positive controls to reconstruct haplotypes. We found that genotypes observed in all carriers of *BRCA1*185delAG* from the San Luis Valley studied here were consistent with the conserved haplotype found in AJ carrier controls. In addition, one non-carrier included in the analysis did not have the conserved alleles at the 7 loci. Our results suggest that individuals from the San Luis Valley likely have at least one Jewish ancestor. In addition, it is possible that some of the de Onate settlers may indeed have been Ashkenazi Jewish. These results are consistent with previous findings which indicate that non-Jewish populations could actually have Jewish ancestors-thus further exemplifying the difficulty in defining ethnicity.

Sequence variants in TLR1, TLR6 and TLR10 are associated with prostate cancer susceptibility. *J. Sun¹, K. Augustsson-Blter², S.L. Zheng¹, M. Hedelin², L. Li¹, H.O. Adami², G. Li¹, J.E. Johnsson⁴, B. Chang¹, D.A. Meyers¹, W.B. Isaacs⁵, J. Xu¹, H. Grnberg³.* 1) Ctr Human Genomics, Wake Forest Univ Sch of Med, Winston Salem, NC; 2) Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; 3) Department of Radiation Sciences, Oncology, University of Ume, Ume, Sweden; 4) Department of Urology and Clinical Medicine, rebro University Hospital, Sweden and Regional Oncological Center, University Hopsital Uppsala, Sweden; 5) Department of Urology, Johns Hopkins Medical Institutions, Baltimore, MD.

Chronic inflammation has been suggested as a causal factor in many human cancers, including prostate. Toll like receptors (TLRs) are essential in triggering innate immunity by sensing the invasion of pathogens as well as cross-talk between the innate and adaptive immunity. Our previous finding of an association of *TLR4* sequence variants and prostate cancer (PCa) risk provides further evidence for a role of TLRs in PCa development. In this study, we hypothesize that sequence variants in the *TLR6*, *TLR1*, and *TLR10* cluster are associated with PCa risk. These three genes reside within a 54 kb region on 4p14 and code for proteins with a high degree of homology in their overall amino acid sequences. The first step of our haplotype tagging SNP (htSNP) approach was to select 33 SNPs covering the three genes and genotype these in 96 Swedish control subjects. Six distinct haplotype blocks were observed and 16 htSNPs could capture >95% haplotype information. These 16 htSNPs were then genotyped in 1,383 newly diagnosed PCa patients and 780 age and residence matched controls. Allele frequencies in 10 of these 16 SNPs were significantly different between cases and controls, with P-values ranging from 0.02-0.003. Two of these htSNPs were nonsynonymous changes in *TLR1*. Haplotype analysis in each block provided stronger evidence. These results provided strong evidence for association of this TLR gene cluster with PCa risk, although it is difficult to dissect which and how many of these positive SNPs confer independent risk. Further functional studies are needed to pinpoint the causal variants in this gene cluster.

Germline sequence variants of NKX3.1 in hereditary prostate cancer families. *S.L. Zheng¹, B.L. Chang¹, G. Li¹, S.D. Isaacs², K.E. Wiley², D.A. Meyers¹, P.C. Walsh², W.B. Isaacs², J. Xu¹.* 1) Ctr Human Genetics, Wake Forest Univ Sch Med, Winston-Salem, NC; 2) Department of Urology, Johns Hopkins Medical Institutions, Baltimore, MD.

NKX3.1, a gene mapped to 8p21, is a member of the NK class of homeodomain proteins and is expressed primarily in the adult prostate. Previous studies suggested that NKX3.1 exerts a growth-suppression and differentiation effect on prostate epithelial cells. Because of known functions and location within a chromosomal region where evidence for prostate cancer linkage and loss of heterozygosity is found, we hypothesize that sequence variants in the *NKX3.1* gene may increase prostate cancer (PCa) risk. To test this hypothesis, we first re-sequenced the promoter, two exons, and exon-intron junctions of the *NKX3.1* gene to identify sequence variants among 188 probands of hereditary prostate cancer (HPC) families recruited at Johns Hopkins Hospital; each family has at least three first-degree relatives affected with PCa. Three non-synonymous changes were identified; including A17T, R52C, and T164A. The A17T and T164 were observed once and in the same subject, who has an African American (AA) background. The R52C variant has been previously reported and was observed multiple times among both European American and AA subjects. In addition, one synonymous change, two variants in the promoter, and 4 variants in the 3UTR were found. We then genotyped all these variants in the 188 HPC families and tested for their co-segregation with PCa risk using both linkage and family-based association tests. Parametric linkage analysis using these 10 variants and one microsatellite marker on each side provided strong evidence for linkage, with a maximum LOD score assuming heterogeneity to be 2.77 ($P=0.0004$) at the 3UTR. For the family-based association tests, five variants, including the three nonsynonymous changes were too rare to be informative. No significant association was found for the remaining variants, suggesting multiple founders (haplotypes) co-segregate with PCa. These results provide evidence that germline sequence variants in NKX3.1 play a role in prostate cancer susceptibility.

Heritable Variation of HER2/ERBB2 as a Determinant of Breast Cancer Histology. *J.P. Breyer¹, J.B. Elmore¹, B.L. Yaspan², Q. Cai⁵, K. Godfrey², K.M. Bradley¹, Y-T. Gao³, D.C. Airey⁴, W. Zheng⁵, J.R. Smith⁵.* 1) Department of Medicine, Vanderbilt University Medical Center, Vanderbilt University, Nashville, TN; 2) Department of Cancer Biology, Vanderbilt University, Nashville, TN; 3) Department of Epidemiology, Shanghai Cancer Institute, Shanghai, PRC; 4) Department of Pharmacology, Vanderbilt University Medical Center, Nashville, TN; 5) Department of Medicine and Vanderbilt-Ingram Cancer Center, Vanderbilt University, Nashville, TN.

Epithelial-derived neoplasms can develop multiple histologic patterns, each with distinct gene expression profiles and clinical sequelae. The epithelial growth factor receptor and ligand family has been broadly implicated in the development and progression of these cancers, particularly adenocarcinoma of the breast. Here we demonstrate that the HER2 member of this gene family is a determinant of breast cancer histology. In a study population of 2503 breast cancer cases and controls from the city of Shanghai, China, the two major population haplotypes of HER2 (frequencies of 0.27 and 0.59) are associated with lobular and ductal carcinoma ($P=0.039$ and $P=0.003$, respectively), the primary histologic types of breast cancer. Further, survival in breast cancer is modified by these haplotypes, the lobular-associated haplotype being relatively protective ($P=0.008$). The same haplotypes predominate in Caucasian populations. The data suggests that common genetic variation at HER2 guides breast carcinoma type, and warrants further investigation of genes in this pathway in common epithelial cancers.

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Genetics study of breast cancer cell lines of MCF_7 (P53+/+) and MDA_MB_468 (P53 mutant) on analyzing replicate genome data of Affymetrix HG-U133A with the design of target MDM2 oligonucleotides, control oligonucleotides and negative control. *K. Chen.* Med Stat S. Hemat/Oncology D., Dept Med; Comprehensive Cancer Center, The U of AL at B'hm, Birmingham, AL.

The study is a genetic identification of the characterized genomes for the two breast cancer cell lines MCF_7 and MDA_MB_468 based on the replicate typing outcome of Affymetrix HG-U133A. The study goal includes the linkage and epistasis content of that the analysis will follow the spatial statistical inference after accomplishing the current genome-wise genetic screening. The per cell line difference in genomes has been doing the 4 comparisons among the three groups of target MDM2 (mouse double minute 2) oligonucleotides (oligo), control oligo and negative control, as well as the between-cell-line differentiation. The presentation is focused on how to manage in analysis for the output of the microarray laboratory with the following defect performance, in addition to the usual bioinformatics issues. Employed Affymetrix HG-U133A of probe_set array of lot 2004204 was typed for target replicates for both cell lines and for the replicates for both controls for mutant cell line, in all 4 array trays; of probe_set array of lot 2004300 was typed two days later for the rest including all original (i.e. non-replicates) for the three cases for both cell lines and replicates for both controls of the wild type cell line, in all 8 array trays.

16q Loss in Retinoblastoma Implicates CDH11 as a Tumor Suppressor Gene. *M.N. Marchong^{1,4}, D. Chen², T.W. Corson^{3,4}, C. Lee⁴, M. Harmandayan⁴, E. Bowles^{1,4}, N. Chen², B.L. Gallie^{1,2,3,4}.* 1) Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada; 2) Department of Ophthalmology, Toronto Western Research Institute, Toronto, ON, Canada; 3) Department of Medical Genetics and Microbiology, University of Toronto, ON, Canada; 4) Department of Cancer Informatics, Ontario Cancer Institute / Princess Margaret Hospital, Toronto, ON, Canada.

Additional genomic alterations accompany loss of both RB1 alleles in retinoblastoma. Previous comparative genomic hybridization (CGH) studies of 50 retinoblastoma revealed 14% genomic loss at chromosome 16q22. We narrowed the minimal region of 16q22 loss to 2.62 Mb by loss of heterozygosity and quantitative multiplex-PCR for sequence tagged site (STS) markers. The highest frequency losses were the STS WI-5835, within intron 2 of the CDH11 gene, and D16S422, within intron 2 of the CDH13 gene. Over 50% of 72 tumors displayed loss within the CDH11 gene, a significantly higher proportion of loss than that detected by CGH. Immunoblot and RT-PCR showed decrease/loss of the intact form of cadherin-11 in 8/14 tumors tested and in 3/8 advanced large TAg-induced murine retinoblastoma (TAg-RB) tumors, while cadherin-13 exhibited no change in expression compared to healthy adult retina. Immunohistochemical analysis of TAg-RB tumors showed that while cadherin-11 is strongly expressed in the inner nuclear layer (INL) of early developing tumors at 4 weeks of age, this expression is lost in foci within late stage tumors at 21 weeks of age. Timing as well as origin of tumor development in the retina is significant due to the fact that retinoblastoma is a developmental disease that arises in the INL of the retina. Our data thus far suggest that CDH11 is expressed in early tumors, but as the tumors progress, deletion of one allele is common, perhaps with mutation of the remaining allele resulting in loss of cadherin-11 protein expression. We have therefore narrowed genomic loss at 16q22 down to a 2.62 Mb minimal region in which one gene, CDH11, exhibits characteristics of a tumor suppressor role in retinoblastoma.

Association of Interleukin-1 receptor antagonist (IL-1Ra) polymorphism in patients with prostate cancer and benign prostatic hyperplasia; A case control study from north India. *R.D. Mittal, D.K. Mishra, H.K. Bid, D.S.L. Srivastava, A. Mandhani.* Department of Urology, SGPGIMS, Lucknow, Uttar Pradesh, India.

Prostate cancer is the most commonly diagnosed noncutaneous cancer. It is a multifactorial and polygenic disease. Different studies suggest that immunological components play a key role in the development of cancers of gastric, cervical and ovary. Polymorphism in IL-1Ra gene has been reported to be associated with different malignant diseases. However, there is scarcity of data depicting role of IL-1Ra in prostate cancer till date. The present study was undertaken to assess the association between prostate cancer and IL-1Ra polymorphism and to evaluate whether frequency of any particular allele has susceptibility to prostate cancer (CaP) and Benign prostate hyperplasia (BPH). In a case control study we analysed the polymorphism of an 86-base pair repeat in intron 2 of the IL-1Ra gene in 96 men with prostate cancer (CaP), 74 with benign hyperplasia (BPH) and 98 healthy controls. Genomic DNA fragments were amplified by polymerase chain reaction. Our study demonstrated a significant dominance of genotype B/B (410 bp) in CaP and BPH patients as compared to normal healthy individuals (57.3% and 56.8% vs 22.4%). However A/A genotype (240 bp) was found to be dominant in normal controls (55.1% vs 6.5% and 16.2%). We found no significant association of IL-1Ra polymorphism with tumor stage and histological grade. Our study suggests that presence of Type B/B genotype may be a risk factor for CaP as well as for BPH while A/A genotype may be protective as observed in healthy individuals. Thus, IL-1Ra gene may be a useful marker for possible screening and risk evaluation in large cohort studies of various populations.

Prostate Cancer and Genetic Variants in *Hepsin* and *CEACAM1*: An Association Study. P. Pal¹, C.H. Jin², R. Kaushal¹, H. Xi¹, G. Sun¹, L. Jin¹, B. Suarez², W.J. Catalona³, R. Deka¹. 1) Dept Environmental Health, Univ Cincinnati, Cincinnati, OH; 2) Dept Psychiatry & Genetics, Washington Univ, St. Louis, MO; 3) Dept Urology, Northwestern Univ Feinberg School of Medicine, Chicago, IL.

Prostate cancer (PC) is the most commonly diagnosed non-cutaneous malignancy among men in the western world. Genes implicated in cell growth, proliferation and differentiation are important candidates in carcinogenesis. We investigated two genes, *Hepsin*, a transmembrane serine protease and *CEACAM1* (carcinoembryonic antigen-related cell adhesion molecule 1), located at 19q2, a region linked with an aggressive phenotype of PC. cDNA microarray studies indicate that *Hepsin* is over-expressed in PC. Down regulation of *CEACAM1* protein in cancer cells is well substantiated. We conducted a case-control association study of PC using 6 SNPs in *Hepsin* and 6 in *CEACAM1*. Four intronic SNPs and one each from 3 and 5 UTR of *Hepsin* were selected. For *CEACAM1*, 3 non-synonymous SNPs located in exon 3, 2 intronic and one located in the 3 UTR were genotyped. The study sample consists of 577 histologically confirmed PC cases from 299 multiplex sibships and 576 unrelated controls, all of Caucasian ancestry. We randomly selected one of the affected sibs from each sibship to form the unrelated cases. Genotype frequencies at all markers were in conformity with Hardy-Weinberg expectations. Minor allele frequencies at two of the *Hepsin* SNPs were <10%, the range at the other four being 25% to 43%. We found significant association with one of the *Hepsin* intronic SNPs (rs2305747, $p = 0.008$), haplotype analysis revealed one major haplotype significantly more prevalent in PC cases ($p = 0.003$; OR = 1.501). At *CEACAM1*, only the three coding SNPs were polymorphic, with minor allele frequencies of ~1% at two and 16% at the third marker. We detected weak association with a minor haplotype at *CEACAM1* ($p = 0.03$). This is the first association study investigating *Hepsin* and *CEACAM1* in PC, and our results suggest that these genes have possible roles in prostate tumorigenesis. Supported by Urological Research Foundation, St. Louis, MO.

Hepatosplenic T-cell lymphoma in a 71-year-old man with X-linked lymphoproliferative disease. *R.D. Klein, M. Ghofrani, A. Bale, T. Duffy, J.G. Howe.* Yale University School of Medicine, New Haven, CT.

X-linked lymphoproliferative disease (XLP) is an X-linked recessive immune deficiency that is characterized by vulnerability to fulminant infections with the Epstein-Barr (EBV) virus. Malignant lymphomas (usually B-cell) and immunoglobulin deficiencies are also observed in the syndrome, most often in association with EBV infection. The disease is caused by mutations in SH2D1A, which is believed to play a role in signal transduction within activated T cells. Affected individuals live to a median age of 10, with few surviving into adulthood. We describe a 71-year-old man with a non-contributory medical history and an apparent XLP mutation who died of a rare T-cell lymphoma. After experiencing a flu-like illness four months earlier, the patient over a six-month period developed persistent fever, fatigue, and weakness, followed by progressive weight loss, anemia, and death from hepatic and renal failure. Diagnostic evaluations during his illness, including liver and bone marrow biopsies and flow cytometry were unrevealing. A limited post-mortem examination attributed the cause of death to hepatosplenic T-cell lymphoma. Serologies demonstrated past infection with EBV, but there was no evidence for EBV infection in the tumor tissue by in situ hybridization with an oligonucleotide probe to EBER-1. During the autopsy, it was found that the patient's 7-year-old grandson had died several years earlier of hepatic failure secondary to an overwhelming EBV infection. The boy had been clinically diagnosed with XLP, although genetic testing was not performed. Sequencing of the SH2D1A gene of both patients revealed a previously undescribed missense mutation, 463G>C. The mutation changes arginine to glutamine at codon 55, a known mutational hotspot. Reported lymphomas in XLP are most often derived from B-cells, and are usually EBV related. The finding of an extremely rare T-cell lymphoma in a 71-year-old man in association with an apparent XLP mutation, indicates a broader phenotype for XLP than has previously been recognized.

A genome-wide scan for linkage disequilibrium based association with breast cancer in an eastern Finnish population. *J. Hartikainen*^{1, 2}, *A. Dunning*³, *H. Tuhkanen*^{1, 2}, *V. Kataja*², *M. Eskelinen*⁴, *V.-M. Kosma*¹, *A. Mannermaa*^{1, 5}. 1) Department of Clinical Pathology and Forensic medicine, University of Kuopio and Kuopio University Hospital, Kuopio, Finland; 2) Department of Oncology, University of Kuopio and Kuopio University Hospital, Kuopio, Finland; 3) Cancer Research UK Human Genetics Research Group, Department of Oncology, Strangeways Research Laboratory, University of Cambridge, United Kingdom; 4) Department of Surgery, University of Kuopio and Kuopio University Hospital, Kuopio, Finland; 5) Department of Clinical Genetics, University of Oulu and Oulu University Hospital, Oulu, Finland.

Breast cancer is the most common of cancers among women in industrialised countries. Many of breast cancer risk factors are known but the majority of the genetic background is still unknown. Linkage disequilibrium based association is a powerful tool for mapping disease genes and is suitable for mapping complex traits in founder populations.

Our aim was to find new genetic medium or low-penetrance breast cancer risk factors in an eastern Finnish population by identifying chromosomal regions that are associated with breast cancer. Our case-control set is from the province of Northern Savo in the late-settlement area of eastern Finland. This population is relatively young and genetically homogeneous. We used 435 autosomal microsatellite markers spaced by an average of 10 cM in a set of 49 breast cancer cases and 50 controls.

In the first-stage scan we found 21 markers in LD with breast cancer (P-values 0.003-0.046 in Fishers exact test). In the second-stage scan with markers flanking 21 positive loci four significant markers were found (P-values 0.013-0.046 in Fishers exact test). Haplotype analysis using global-score method with two, three or four markers also revealed four positive marker combinations (simulated P-value for global score 0.003-0.021). Our results suggest four breast cancer associated regions in three chromosomes in an eastern Finnish population.

Failure to Replicate Linkage of Hereditary Prostate Cancer to 20q13 in the ICPCG Family Collection. *B. Chang*¹, *D. Schaid*², *The International Consortium for Prostate Cancer Genetics*. 1) Ctr Human Genomics, Wake Forest Univ Sch Med, Winston-Salem, NC; 2) Division of Biostatistics, Mayo Clinic, Rochester, MN.

The International Consortium for Prostate Cancer Genetics (ICPCG) is an international collaborative effort to pool pedigrees with hereditary prostate cancer in order to replicate linkage findings for prostate cancer. A strength of the ICPCG is the large number of well-characterized pedigrees, allowing linkage analyses within large subsets. Given the heterogeneity and complexity of prostate cancer, the historical difficulties of synthesizing different studies reporting positive and negative linkage replication, and the use of different statistical analysis methods and different stratification criteria, the ICPCG provides a valuable resource to evaluate linkage for hereditary prostate cancer. To date, linkage of chromosome 20 (HPC20) to hereditary prostate cancer has been one of the strongest linkage signals, yet the efforts to replicate this linkage have been limited. This paper reports a linkage analysis of chromosome 20 markers for 1,234 pedigrees with multiple cases of prostate cancer ascertained through the ICPCG, and represents the most thorough attempt to confirm or refute linkage to chromosome 20. From the original 158 Mayo pedigrees in which linkage was detected, the maximum heterogeneity lod score, under a recessive model, was 2.78. In contrast, for the 1,076 pedigrees not included in the original study, the maximum heterogeneity lod score (recessive model) was 0.06. Although a few small linkage signals for chromosome 20 were found in various strata of this pooled analysis, in general this large study failed to replicate linkage to HPC20. This study illustrates the value of the ICPCG family collection to evaluate reported linkage signals and suggests that the HPC20 region does not make a major contribution to prostate cancer susceptibility. (Supported by PHS U01-CA89600).

Detection of ultra rare somatic mutations in the p53 gene by Bi-PAP-A. *J. Shi, Q. Liu, S.S. Sommer.* Department of Molecular Genetics and Molecular Diagnosis, City of Hope National Medical Center, Duarte, CA 91010-3000.

Ultra sensitive detection of somatic mutations can facilitate: i) early cancer detection; ii) cancer chemotherapy; and iii) assessment of environmental mutagen exposure. Bi-PAP-A (**B**i-directional **P**yrophosphorolysis **A**ctivated **P**olymerization **A**llele-specific Amplification) can detect mutations at 1 part per billion. The sensitivity of Bi-PAP-A derives from the serial coupling of pyrophosphorolysis and DNA polymerization. Eight Bi-PAP-A assays were developed for the p53 gene. Based on the IARC TP53 mutation database, four of them were hotspots of mutation in tumors. The sensitivity (minimum copies of the mutated template with a detectable mutated product), and specificity (maximum copy number of the wild-type template with undetectable product) of each assay were tested using mutated and wild type human genomic DNA extracted from blood samples. Low levels of somatic mutations were found at three assays that are not hotspots of mutation in tumor, indicating the frequency of somatic mutation in these samples was lower than 3 parts in 10^7 . For three of the assays, including two of the hotspots in tumors, the mutation frequency was approximately 1 part in 10^6 . For the remaining two assays, which were hotspots of mutation in p53 gene, frequency of somatic mutation was greater than 1 part in 10^4 . Inter-individual variation in mutation frequency can be dramatic, greater than 14 fold in some assays. The marked inter-locus and inter-individual variations in somatic mutation frequencies in the p53 gene have implications for human carcinogenesis. Bi-PAP-A is a convenient and general method that has the desirable properties of allele-specific PCR with a sensitivity that is typically enhanced by a million fold.

Complex segregation analysis and linkage mapping of nasopharyngeal carcinoma in Guangdong, China. *Y. Yao¹, W. Jia³, A. Collins², B.J. Feng³, X.J. Yu³, L.X. Huang³, Q.S. Feng³, P. Huang³, M.H. Yao⁴, F. Zhang³, W. Huang⁵, Y.X. Zeng³.* 1) Dept Epidemiology, Johns Hopkins Univ, Baltimore, MD, USA; 2) 3Human Genetics, University of Southampton, Southampton General Hospital, UK;; 3) Departments of Experimental Research , Cancer Center, Sun Yat-sen University, GuangZhou, China;; 4) Departments of Information, Cancer Center, Sun Yat-sen University, GuangZhou, China;; 5) Chinese National Human Genome Center at Shanghai, Shanghai, China.

The purpose of this study is to investigate the fit of single gene, polygenic and multifactorial models to the observed pattern of transmission of NPC in a hospital-based family history study conducted by the Cancer Center of Sun Yat-Sen University (CCSYU) in Guangzhou, China. Complex segregation analysis of a total of 1,903 Cantonese families ascertained at CCSYU was conducted using a unified mixed model after the pedigrees were partitioned into 3,737 nuclear families. The mixed model assumes that a phenotype is influenced by the additive and independent effect of a major gene, together with a multifactorial component (genetic and environmental) and a random environmental effect. Segregation analysis results do not provide strong evidence for a major gene and the observed data is best explained by a multifactorial mode of inheritance for NPC. We also performed a genome scan on 32 multiplex NPC families. A linkage signal was detected by parametric linkage analyses on chromosome 4 with a LOD score of 3.06 and HLOD of 3.21 at marker D4S405. Fine scale mapping with additional markers flanking D4S405 resulted in a LOD of 3.92 and HLOD of 3.99 for the region 4p15.1-q12. Furthermore, multipoint NPL analysis gave a LOD score of 3.54 at D4S405 and 4.2 at D4S3002, which is 4.5 cM away from D4S405. Our previous analyses indicated that a susceptibility locus on 4p15.1-q12 may account for a subset of hereditary NPC by linkage analysis although the location of the gene may not be precise due to limited sample size. Covariate-based linkage analysis will be performed to identify the source of heterogeneity. Information EBV infection and age of onset included in the model.

Methylation status of hMLH1 repair gene promoter in esophageal pathologies including cancer. *Y.R. Ahuja^{1,2}, M. Vasavi³, V. Bhavani⁴, B. Prabhakar⁵, P. Rupender⁴, B. Ravi Shankar³, S. Radha³, Q. Hasan^{3,4}*. 1) VMRC, Vasavi Hosp, Hyderabad, India; 2) Stem Cell & Mol Biol Lab, NIMS, Hyd, India; 3) Depts of Genetics & Mol Med, GE & Pathol, Kamineni Hosp, Hyd, India; 4) Depts of Genetics & Dept of GE, BMMRC, Hyd, India; 5) Dept of GE, Osmania Gen Hosp, Hyd, India.

More than 30 percent of the adult population suffers from esophageal pathologies. Esophageal cancer is one of the most fatal cancers in the world. Cancer may arise either by gene mutations or by epigenetic events like methylation that alter the gene expression status. The mismatch repair (MMR) system maintains genomic integrity and defects in MMR genes are responsible for genetic instability. Promoter hypermethylation of hMLH1 has been associated with increased genetic instability. The aim of this study was to assess the methylation status of the repair gene hMLH1 in esophageal cancer and other inflammatory pathologies of the esophagus. 67 individuals referred for endoscopy of the upper GI tract were enrolled in the study. Endoscopic biopsy material was sent for histopathological analysis and for molecular analysis. DNA extracted from biopsy was subjected to HpaII restriction digestion, amplified using primers specific for hMLH1 promoter by methylation specific PCR assay (MSP). Individuals were categorized by endoscopy and histopathology into cancer (30), esophagitis (23) and normal (14). MSP assay showed a hypermethylated hMLH1 promoter in 66.7% of cancer cases, 56.5% of cases with different grades of esophagitis and 21.4% of cases with dyspepsia with normal endoscopy. Chi-square test showed that hMLH1 hypermethylation was significantly higher in cancer and esophagitis compared to endoscopically normal tissue ($p < 0.005$, $p < 0.03$ respec). However, there was no significant difference between cancer and esophagitis. hMLH1 hypermethylation is known to cause defective DNA MMR resulting in genetic instability. Our results indicate that altered methylation at hMLH1 locus is not confined to esophageal cancer but is also seen in non-malignant inflammatory pathologies of the esophagus, suggesting that factors effecting repair are not cancer specific.

Analysis of epigenetic changes in *MGMT* gene in retinoblastoma tumors. *P. Kadam*¹, *Y.F.L. Ling*², *P.S. Lai*¹. 1) Paediatrics, National University Singapore, Singapore, Singapore, Singapore; 2) Singapore National Eye Centre, Singapore.

Retinoblastoma is a malignant childhood tumor caused by inactivation of *RB1* gene. *RB1* gene is commonly inactivated by point mutations and chromosomal mechanisms that result in loss of heterozygosity (LOH). Besides this, hypermethylation of the CpG rich island of *RB1* gene promoter has also been reported in retinoblastoma tumors. Recent studies have shown that other candidate genes may also be involved in tumorigenesis in retinoblastomas. The DNA repair gene, *MGMT* has been reportedly silenced by promoter hypermethylation in a number of cancers such as gliomas, non-small cell cancer of the lung, colorectal cancers and lymphomas. In this study, we investigated the presence of hypermethylation of *MGMT* promoter in retinoblastoma tumors in patients from Singapore. A series of 36 tumors was studied by methylation-specific PCR (MSP) analysis. Aberrant methylation of at least one *MGMT* locus was found in 9 (25%) retinoblastoma tumors which included 4 hereditary and 5 non-hereditary cases. Of the methylated samples, LOH in *RB1* locus was present in 7 tumors and point mutations were found in 8 tumors while hypermethylation of *RB1* promoter was found in 1 tumor only. We also investigated the presence of G>A transitions in *RB1* gene as previous reports have suggested that epigenetic silencing of *MGMT* may lead to this type of mutagenic effect. G>A transitions in *RB1* were found in 5 (55.5%) of tumors hypermethylated at *MGMT*. No significant correlation was found between *MGMT* hypermethylation and age of onset, tumor types or genetic mode of origin of disease. Thus, this study suggest that hypermethylation of *MGMT* is involved in retinoblastoma development but its occurrence does not seem to be preferentially associated with any inherited disease genotype or clinical phenotype.

Characterization of Cytosine Methylation in Lung Cancer using MALDI-TOF mass spectrometry. *D. van den Boom*¹, *M. Nelson*¹, *T. Liloglou*², *J. Field*², *M. Ehrich*¹. 1) Dept Molecular Biology, SEQUENOM, Inc, San Diego, CA; 2) Roy Castle Lung Cancer Research Programme, The University of Liverpool Cancer Research Centre, Liverpool, UK.

Methylation is considered to be one of the major epigenetic processes pivotal to our understanding of carcinogenesis. It is now widely accepted that there is a relationship between DNA methylation, chromatin changes and human malignancies. DNA methylation has the potential to become an important clinical marker in cancer molecular diagnostics. We applied a novel method for high throughput DNA methylation analysis that utilizes MALDI-TOF mass spectrometry (MS) analysis of base-specifically cleaved amplification products for the characterization of cytosine methylation differences between normal and neoplastic lung cancer tissue. In a candidate gene approach, we selected 47 CpG islands covering over 1426 CpG positions and compared the relative methylation ratio in 48 non-small cell lung cancer (NSCLC) samples with their corresponding adjacent normal tissue. Our study demonstrates that base-specific cleavage analyzed by MALDI-TOF MS provides an elegant means to identify methylated sites in a high-throughput fashion. At the same time, the method allowed us to quantitate the ratio of methylation at each CpG individually or at groups of CpGs. We will show that hierarchical clustering based on these methylation ratios allows separation of tumor samples and normal tissue. Furthermore, we applied tree-based procedures to isolate CpG positions that allow for classification of samples with high accuracy.

Correlation of CHEK2 Protein Expression and 1100delC Mutation Status with Tumor Characteristics among Unselected Breast Cancer Patients. *O. Kilpivaara¹, J. Bartkova², H. Eerola^{1, 3}, K. Syrjäkoski⁴, P. Vahteristo¹, J. Lukas², C. Blomqvist^{3, 5}, K. Holli⁶, P. Heikkilä⁷, G. Sauter⁸, O.-P. Kallioniemi⁹, J. Bartek², H. Nevanlinna¹.* 1) Dept. Obstetrics & Gynecology, Helsinki University Central Hospital (HUCH), Helsinki, Finland; 2) Inst. Cancer Biology, Danish Cancer Society, Copenhagen, Denmark; 3) Dept. Oncology, HUCH; 4) Lab. Cancer Genetics, Inst. Medical Technology, Tampere University and Tampere University Hospital, Finland; 5) Dept. Oncology, Uppsala University Hospital, Sweden; 6) School of Medicine, Tampere University and Tampere University Hospital, Finland; 7) Dept. Pathology, University of Helsinki; 8) Inst. Pathology, University of Basel, Switzerland; 9) Medical Biotechnology, VTT Technical Research Centre of Finland and University of Turku, Finland.

The CHEK2 kinase is a tumor suppressor whose activation in response to DNA double strand breaks contributes to cell cycle arrest or apoptosis. The 1100delC mutation is associated with familial breast cancer, and tumors from mutation carriers show reduced or absent CHEK2 protein expression. We have here studied CHEK2 protein expression by immunohistochemistry on a tissue microarray of 611 unselected breast tumors, and also evaluated the tumor characteristics among 1297 unselected breast cancer patients defined for the 1100delC germ line mutation status (2.5% carrier frequency). CHEK2 protein expression was reduced in 21.1% of the unselected breast cancers studied. Tumors with reduced CHEK2 expression had more often larger primary tumor size (pT3-pT4, $p=0.002$) compared to tumors with normal staining. A similar trend for larger tumor size was seen among the 37 breast tumors from 1100delC germ line mutation carriers. Tumors from 1100delC mutation carriers were of higher grade than those of non-carriers ($p=0.02$). The 1100delC germ line mutation also associated strongly with bilateral breast cancer. No significant correlation was seen between CHEK2 status and hormone receptor status, histology, lymph node status, or overall survival. The reduction or loss of CHEK2 function may contribute to progression of breast carcinomas.

Association between sequence variation at the human Frizzled-1 (*FZD-1*) locus and prostate cancer. C.H. Bunker¹, S.P. Moffett¹, J.P. Oakley¹, C.S. Nestlerode¹, A.L. Patrick², J.M. Zmuda¹. 1) University of Pittsburgh, Pittsburgh, PA; 2) Tobago Health Studies Office, Scarborough, Tobago.

Inappropriate activation of the canonical Wnt/-Catenin signaling pathway has been identified as an important feature in human neoplasia. Canonical Wnt-signaling is mediated through a family of integral membrane receptors known as Frizzleds, which activate target gene transcription via downstream -Catenin. The Frizzled-1 mRNA is highly expressed in adult prostate and the *FZD-1* locus maps to human chromosome 7q21, where modest evidence for linkage to hereditary prostate cancer has been reported (M. Janer et al., *The Prostate* 57:309, 2003). We directly sequenced 3.3 kb of the human *FZD-1* locus using genomic DNA extracted from peripheral blood mononuclear cells from 24 men with prostate cancer and 24 negative controls. All men were participants in the Tobago Prostate Survey, an ongoing population-based screening study of Afro-Caribbean men between the ages of 40-79 years on the island of Tobago. To date, 3083 of 5121 eligible men in the population have been screened with serum prostate specific antigen (PSA) and digital rectal examination (DRE) yielding a 10% prevalence of screening detected prostate cancer. We identified a CCG (Proline) repeat (range 4-5 repeats) at Codon 89, and 23 single nucleotide polymorphisms (SNPs). Fifteen of the SNPs were located within a 650bp region upstream from translation start site. In preliminary genetic association analyses of the most common allelic variants (minor allele frequency 0.04), four of the five common variants showed statistically significant evidence (P<0.05) of allele frequency difference between cases and controls. Three of these four variants were located in the putative 5' promoter region. Five proline repeats were observed in 56% of cases compared with 34% of controls (p<0.05). In conclusion, we have identified several common allelic variants at the human *FZD-1* locus. Preliminary analyses suggest a novel association with prostate cancer in men of African origin. Further studies with larger sample size and additional markers within and flanking the *FZD-1* locus are needed to confirm these results.

Detection of large rearrangement mutations in *BRCA1* and *BRCA2* in 528 high risk families from North America by quantitative PCR based gene dose analysis. *T. Judkins, B.C. Hendrickson, D. Gonzales, K. Eliason, J. McCulloch, B.E. Ward, T. Scholl.* Myriad Genetic Laboratories, Salt Lake City, UT.

Large rearrangement mutations are estimated to account for 5-10% of all mutations in *BRCA1* and *BRCA2*. For point mutations in the *BRCA* genes, North American hereditary breast/ovarian cancer patients carry a broader spectrum of mutations in comparison with many European populations where founder mutations can predominate. Prevalent founder rearrangement mutations have also been described in European patients. Here, the spectrum and prevalence of rearrangement mutations in the *BRCA* genes was determined in a set of high risk North American patients. Five hundred twenty-eight anonymous specimens that were negative by clinical direct DNA sequencing of *BRCA1* and *BRCA2* were selected for study. These patients were affected with breast or ovarian cancer before fifty years of age and reported at least two family members affected with cancer. Genomic DNA from these patients was analyzed by multiplexed quantitative PCR assays to determine gene dose across both *BRCA1* and *BRCA2* by interrogating all exons and the promoters of both genes. Approximately 6% of patients carried rearrangement mutations, corresponding to approximately 14% of all mutations when mutations detected by sequencing are included for patients similarly stratified. The most common rearrangement detected was a *BRCA1* duplication of exon 13 which accounted for over one-third of all the rearrangements detected. This particular mutation is known to be prevalent in patients with various ancestries. Novel rearrangement mutations were among others detected and included the duplication of exons 5-13 in *BRCA1*, the complete deletion of *BRCA1*, and the deletion of exons 1-23 of *BRCA1*. These results concur with previous reports on the prevalence of rearrangement mutations.

Macronodular adrenocortical hyperplasia associated with an inactivating mutation of the fumarate hydratase gene and loss of its 1q42.3-43 locus. L. Matyakhina¹, R.J. Freedman^{1, 2}, I. Bourdeau¹, A. Chidake², M. Walther³, J. Toro⁴, W.M. Linehan³, C.A. Stratakis^{1, 2}. 1) Developmental Endocrinology Branch, NICHD, Bethesda, MD; 2) Inter-Institute Endocrinology Training Program, NICHD, Bethesda, MD; 3) Surgical Urology Branch, NCI, Bethesda, MD; 4) Section on Electron Microscopy, Laboratory of Pathology, NCI, Bethesda, MD.

Hereditary leiomyomatosis and renal cell cancer (HLRCC) is an autosomal dominant disorder characterized by cutaneous and/or uterine leiomyomas, occasional leiomyosarcomas, renal cancer, and possibly other tumors. Recently, mutations in the fumarate hydratase gene (*FH*), on chromosome 1q42.3-43, have been found to be responsible for this disorder in a number of kindreds. Massive macronodular adrenocortical disease (MMAD) or ACTH-independent macronodular adrenal hyperplasia (AIMAH) is a heterogeneous condition associated with Cushing syndrome (CS) and bilateral enlargement or adenomas of the adrenal glands. In MMAD, cortisol secretion is often mediated by gastric inhibitory polypeptide (GIP), vasopressin, catecholamines, and other hormones due to aberrant, adrenocortical expression of their receptors. Although this mechanism may underlie pathophysiology of CS in the majority of CS cases, no consistent genetic defects have been identified in the few families with MMAD that have been reported. We report the case of a patient with HLRCC with a novel, inactivating *FH* mutation, who presented with atypical, periodic CS due to bilateral, ACTH-independent adrenocortical hyperplasia. Clinical and histological investigations showed features consistent with MMAD. The tumor tissue harbored the germline *FH* mutation and demonstrated allelic losses of the 1q42.3-43 *FH* locus. We conclude that in an HLRCC patient bilateral adrenal enlargement and CS were due to MMAD. This pathology was most likely due to the *FH* germline mutation because in tumor cells only the mutant allele was retained, consistent with the Knudsons hypothesis and *FH*'s tumor suppressor role. HLRCC should be added to the growing list of monogenic disorders that may be associated with a genetic predisposition to the development of adrenocortical tumors.

Evaluation of *RAD51* alterations and *BRCA2* BRC variants in hereditary susceptibility to breast and ovarian cancer. *K. Rapakko, S.-M. Karppinen, K. Heikkinen, R. Winqvist.* Dept Clinical Genetics, University of Oulu/OYS, Oulu, Finland.

Breast and ovarian cancer are among the most frequent malignancies affecting women, and about 5-10% of the cases appear to be due to inherited mutations in various cancer susceptibility genes. *BRCA1* and *BRCA2* mutations are particularly important in families with a large number of early-onset cancers, but play a much lesser role in smaller cancer families and in families without cases of ovarian cancer, thus indicating involvement of additional cancer susceptibility genes. In addition, variation in disease penetrance has frequently been observed among *BRCA1* and *BRCA2* mutation carriers, suggesting the influence of cancer risk modifying factors.

BRCA2 regulates the activity of *RAD51*, the human homolog of *E. coli* RecA. The interaction occurs through the conserved BRC repeat motifs of *BRCA2*. The *BRCA2-RAD51* complex is essential for homology-dependent recombinational repair of DNA double-strand breaks that occur frequently in dividing cells. Erroneous repair of these lesions can lead to cancer. In the current study we have screened 192 patients of 129 Finnish breast and ovarian cancer families for possible cancer associated germline mutations in the *RAD51* gene, and also specifically in the BRC motifs of the *BRCA2* gene. Furthermore, we have elucidated the role of the previously described 135G>C single-nucleotide polymorphism in the 5 untranslated region of *RAD51* on cancer predisposition.

Mutational Analysis of HPC1/RNASEL and the Macrophage Scavenger Receptor 1 Genes in Asian-Indian Sporadic Prostate Cancer and Benign Prostatic Hyperplasia. *H. Rennert¹, C. Sadowl¹, M.J. Finley¹, R. Mittal², A. Mandhani², T.R. Rebbeck¹, B. Mittal².* 1) University of Pennsylvania, Philadelphia, PA; 2) Sanjay Gandhi Post Graduate Institution of Medical Sciences, Lucknow, India.

The HPC1/RNASEL gene on 1q25 and the Macrophage Scavenger Receptor1 (MSR1) on 8p22 were recently reported as candidate genes for hereditary prostate cancer. We evaluated the role of these genes in prostate cancer risk in the Asian-Indian population. DNA samples from 90 Asian-Indian patients with sporadic prostate cancer and 58 patients with benign prostatic hyperplasia as well as 47 age-matched controls were genotyped using the Wave (Transgenomic) DHPLC system and sequencing. In RNASEL, a total of 8 variants were identified including, 4 exonic (151insCAAT, 1078A>G, 1260C>T and 1623T>G) and 4 intronic (IVS1+59A>G, IVS4+4T>C, IVS5-27T>C and IVS6+47G>A) changes. The 4-bp insertion mutation, 151insCAAT, at codon 151 in RNASEL exon 1, results in a premature truncation at codon 157. This mutation was present in approximately 1% of the study subjects. Of the 4 exonic changes, one previously reported common missense change (Asp541Glu) was identified. The common RNASEL missense variant, Arg462Gln, was not identified in this study. In MSR1, a total of 7 variants were identified including, 4 exonic (171C>T, 542T>A, 732G>A and 823C>G) and 3 intronic (5UTR-33A>G, IVS2-10G>A and IVS7+36G>A) changes. Of the 4 MSR1 exonic changes, only Pro275Ala was previously reported. No deleterious mutations were detected in MSR1. These results demonstrate that sequence variants in these genes also exists in Asian-Indian populations, however, their pattern and distribution is different than those reported for other populations. Further studies, however, are needed to determine their role in prostate cancer etiology in Asian-Indians.

Identification of NF1 Gene Mutations Using Universal Microarray-based Combinatorial Sequencing-by-Hybridization. A. Schirinzi^{1,2}, S. Drmanac³, S. Huang³, D. Swanson³, R. Drmanac³, A. De Luca², B. Dallapiccola², S. Surrey¹, P. Fortina¹. 1) Department of Medicine, Thomas Jefferson University, Philadelphia, PA; 2) Dipartimento di Medicina Sperimentale e Patologia, Universita' La Sapienza and Istituto CSS Mendel, Rome, Italy; 3) Callida Genomics, Sunnyvale, CA, USA.

Neurofibromatosis type 1 (NF1) is a common autosomal dominant disorders occurring with a frequency of 1 in 3500 individuals. The disease results from mutations in the NF1 gene which encodes neurofibromin, a negative regulator of the ras-dependent pathway. Mutation detection presents a considerable challenge due to the large size of NF1 gene and the presence of pseudogenes. Here we demonstrate a method to detect mutations in the NF1 gene using an array-based approach referred to as combinatorial sequencing-by-hybridization (cSBH). A universal probe set (i.e., all possible 6-mers) is arrayed on a glass slide and a second probe set (i.e., all possible 5-mers) is in solution and labeled with a fluorophore. Ligation occurs only when a complementary DNA strand from the patients PCR product anneals to both array-bound and solution phase, labeled probe. After ligation, 11 nt long positive labeled probes are created and detected using a standard arrayer which scores fluorescent signals at each register. Software then deduces the DNA sequence by a tiling approach in which the same regions are sequenced in multiple overlapping 11-mer probes. We generated 25 PCR products of 150-584 bp from patient DNA to confirm mutations previously identified by DHPLC and direct DNA sequence analysis. Furthermore, using cSBH, we analyzed the entire gene coding region (~12 kb) by pooling of PCR products encompassing the 60 exons from the NF1 gene. Continuous sequence readout of up to 3.6 kb was obtained on a single slide. Using this approach, we confirmed NF1 gene mutations in all 25 patient samples. The universal chip used for cSBH successfully detects base substitutions, insertions, and small deletions. Also, by using cSBH, analysis of the entire NF1 gene is fast and efficient, reducing time and cost. cSBH is a viable alternative to current techniques used in many diagnostic laboratories requiring DNA sequence characterization.

Profiling multigenic variation in androgen pathway as a risk factor for prostate cancer. E. Seppala¹, N. Mononen¹, T. Ikonen¹, V. Autio², P. Ellonen³, J. Saharinen³, J. Saarela³, M.P. Matikainen⁴, T.L.J. Tammela⁴, O. Kallioniemi⁵, J. Schleutker¹. 1) Lab of Cancer Genetics, Inst Medical Technology, Univ of Tampere and Tampere University Hospital, Finland; 2) Research Unit, Tampere Univ Hospital, Finland; 3) Dept of Molecular Medicine, National Public Health Inst, Helsinki, Finland; 4) Div of Urology, Tampere Univ Hospital and Medical School, Univ of Tampere, Finland; 5) Technical Res Centre and Univ Turku, Finland.

In order to study the impact of genetic variation along the androgen pathway on prostate cancer (prca) risk we have so far screened eleven candidate genes (*SRD5A2*, *CYP17*, *CYP19*, *HSD3B1*, *HSD3B2*, *HSD17B2*, *HSD17B3*, *HSD17B5*, *CYP11A*, *KLK3*, *ARSDR1*) for germ line mutations and polymorphisms using 64 DNA samples from Finnish prca patients by SSCP. In previous reports, many genes along this pathway have been associated with prca risk but the analyses have been carried out in rather limited sample sets. Here, the found variants were genotyped in an extensive set of 1892 samples using either allele specific primer extension on micro array or TaqMan 5nuclease assay. The allele frequencies of the variants are compared in cases of unselected prca (n=848, diagnosed in Pirkanmaa district in 1999-2001), familial prca (n=121) and population controls (n=923). Our main goal is to find out the multivariant joint effects. Preliminary results from separate analyses of the *HSD17B5* gene variants suggested that P180S may have a protective role (OR=0.7, p=.060) whereas Q5H did not associate with the cancer risk (OR=0.9, p=.375). Likewise, no statistically significant association between prca risk and any of the other variants tested so far (*SRD5A2* V89L and A49T, *LHB* I15T, *AR* R726L, *CYP17* -34T>C, *HSD17B2* A111T or *KLK3* D102N) was observed. However, the T201M variant of the *CYP17* gene did associate with prca risk (controls *versus* unselected cases, OR= 2.0, p=.04). We also tested the joint effect of the two *SRD5A2* variants by comparing the frequencies of AA/LL genotype *versus* AT+TT/VL+VV among the unselected prca cases (n=80, n=40, respectively) and controls (n=82, n=46, respectively) but no statistically significant effect was observed (p= 0.7).

Population based study of HNPCC mutations in Newfoundland. *P.A. Williams¹, M.O. Woods¹, R.C. Green¹, J.R. McLaughlin², P.S. Parfrey³, H.B. Younghusband¹*. 1) Genetics, Memorial University, St. John's, NL, Canada; 2) Mount Sinai Hospital, Toronto, ON, Canada; 3) PRC, Memorial University, St. John's, NL, Canada.

Hereditary Non-Polyposis Colorectal Cancer (HNPCC) is an autosomal dominant condition that increases an individual's lifetime risk of developing cancer. HNPCC is caused by mutations in genes in the Mismatch Repair (MMR) pathway. Five genes have been found to contain HNPCC causing mutations - *MLH1*, *MSH2*, *MSH6*, *MSH3*, and *PMS2*. Mutations in *MLH1*, *MSH2* and *MSH6* account for 95% of HNPCC cases. The Newfoundland Colon Cancer Registry (NFCCR) was created to accumulate molecular, as well as family history data, for the population of Newfoundland for the purpose of identifying those who are at high risk for hereditary cancer. All colorectal cancer patients diagnosed from 1999 to 2003 were invited to participate in the registry. Upon completion the registry will consist of approximately 600 probands, with accompanying molecular data. All participants have had a DNA sample banked and their tumours have been characterized for microsatellite instability (MSI), a hallmark feature of HNPCC. As well, immunohistological data for *MLH1*, *MSH2* and *MSH6* has been obtained. The risk status of the patients was determined as high risk if they fulfilled any of the following criteria: 1) belonging to a high family risk category such as Amsterdam I or Amsterdam II, 2) tumour samples which had deficient staining for one of the three MMR proteins listed above, or 3) tumour samples displaying MSI. Those identified as high risk were then subjected to mutation analysis. Samples were initially tested for large genomic deletions in *MLH1* and *MSH2* via Multiplex Ligation-dependent Probe Amplification (MLPA). Samples negative for a deletion were tested for the *MSH2* IVS5+3AT mutation, common in the Newfoundland population, by Restriction Fragment Length Polymorphism. Samples showing no mutation for the first two methods were then screened for mutations by direct sequencing of *MLH1*, *MSH2* and *MSH6*. To date, 113 samples have been tested via MLPA, and have shown no deletions. One of the samples had the *MSH2* IVS5+3AT. Currently, automated sequencing has been undertaken for *MLH1*, *MSH2*, and *MSH6*.

High Resolution Analysis of OSCCs Using the Whole Genome SMRT Array. *C.S.M. Baldwin¹, C. Garnis¹, M.P. Rosin¹, L. Zhang², W.L. Lam¹.* 1) Cancer Genetics, BC Cancer Research Centre, Vancouver, BC; 2) 2Department of Oral Biological & Medical Sciences, University of British Columbia, Vancouver, BC.

Each year, there are ~300,000 cases of oral squamous cell carcinoma (OSCC) world-wide. Most cases are not diagnosed or treated until the advanced stages of disease, resulting in a 5-year survival rate of just 50%. If diagnosed at the pre-malignant stages, prognosis and survival rates improve. Identification of novel genetic alterations in OSCCs will provide new targets for early diagnosis and treatment. LOH studies on OSCCs have identified the earliest genetic alterations to occur on 9p, 3p and 17p. However, LOH is not designed to distinguish copy number gains from losses and the resolution is limited. Conventional CGH, although able to distinguish between copy number increases and decreases, also has limited resolution. We present a new genome-wide approach to fine-map alterations with ~80kb resolution to identify novel genes involved in OSCC. **OBJECTIVE:** The objective of this study is to detect novel regions of alteration in OSCCs using our high resolution human whole genome BAC array CGH technology in order to elucidate the genetic mechanisms underlying OSCC. **METHODS:** We utilized the Submega Base Resolution Tiling-set (SMRT) array comprised of 32,433 over-lapping BAC clones spanning the entire human genome. DNA from each BAC clone was PCR-amplified and spotted in triplicate onto two glass slides. OSCC DNA and normal reference DNA were labeled with cyanine-dyes, co-hybridized onto the SMRT array, and scanned for each dye to determine the hybridization signal intensity ratios for each loci. The data was plotted to reveal copy number gains and losses across the entire human genome. **CONCLUSION:** We profiled 20 OSCCs using the SMRT array. Alignment of genomic profiles revealed recurrent known (3p, 9p, 11q, 7p) and novel alterations. Genome profiling OSCCs using the SMRT array revealed novel and known copy number alterations. Further expression analysis will elucidate the biological mechanisms underlying OSCC progression. This work was supported by funds from Genome Canada/BC, CIHR and NICDR.

Screening for germline mutations in the *PTEN*, *MSR1* and *KLF6* genes in prostate cancer patients. A. Bar-Shira¹, H. Matzkin^{2,3}, N. Matarasso^{1,3}, S.D. Bercovich¹, A. Orr-Urtreger^{1,3}. 1) Genetic Institute; 2) Department of Urology; Tel Aviv Sourasky Medical Center; 3) Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

Prostate cancer is the most commonly diagnosed malignancy in men in the western world, including Jewish men in Israel, and one of the leading causes of cancer-related death. Multiple risk factors including age, race, environmental exposure and a positive family history contribute to the high incidence of prostate cancer. Despite the sporadic nature of this disease, 10-15% of men with prostate cancer have one or more affected first-degree relatives. At least ten genome-wide linkage scans in hereditary prostate cancer families have revealed prostate cancer-susceptibility chromosomal loci possibly harboring candidate hereditary prostate cancer genes. Mutations in these genes likely account for 9% of prostate cancer diagnosed in men through age 85. The Jewish population, with its unique structure and origin, has been most valuable for the identification of cancer predisposition mutations. All cancer-susceptibility founder mutations detected in Jews were identified in genes associated with cancer in the population-at-large. *PTEN*, *MSR1* and *KLF6* genes were recently suggested as candidate susceptibility genes for hereditary and sporadic prostate cancer. In order to identify germline mutations and polymorphisms in these genes, DNA from 300 Ashkenazi and non-Ashkenazi Jewish prostate cancer patients was screened using DHPLC. The sequence of the *MSR1* gene was intact in all patients. Two sequence variations were detected in the *PTEN* gene, a missense and a silent mutation. In the *KLF6* gene, five changes were detected in 17 patients, including a pre-mature stop codon and a missense mutation. Our results suggest that germline mutations in the *MSR1* and *PTEN* genes do not play a role in hereditary susceptibility to prostate cancer in Jews, and that mutations in *KLF6* may occasionally be found in these patients. These results further emphasize the complexity of the genetic background for prostate cancer pathogenesis.

Extending the basal phenotype of BRCA1-related breast cancer: relevance for the re-classification of breast cancer. *J.S. Brunet^{1,2}, J. Arnes³, T. Nielsen⁴, D. Huntsman⁴, N. Wong⁵, L.R. Bgin⁶, L.A. Akslen³, W.D. Foulkes^{1,5}.* 1) Prog. in Cancer Genetics, McGill Univ. Montreal, QC, Canada; 2) Algorithmic Pharma, Laval, QC, Canada; 3) The Gade Institute, Haukeland University Hospital, Bergen, Norway; 4) Div. Medical Genetics and BC Cancer Agency, Univ. British Columbia, Vancouver, BC, Canada; 5) Cancer Prevention Centre, SMBD-Jewish General Hospital, Montreal, QC, Canada; 6) Sacre Coeur Hospital, Cartierville, QC, Canada.

BRCA1-related breast cancers are associated with the expression of cytokeratin (CK5/6), thus such tumours could be described as basaloid. Other markers e.g. cyclin E, p27 and glomeruloid microvascular proliferation are also associated with both CK5/6 expression and BRCA1 mutations and all three are independent adverse prognostic factors following breast cancer. In this study, we extended the basal phenotype by studying three other markers: EGFR, P-cadherin and MDM2. We used our existing retrospective cohort of 292 Ashkenazi Jewish breast cancer patients who have been tested for BRCA1 mutations. For EGFR and MDM2, a tissue microarray of 230 cases was used, for P-cadherin (P-cad), standard slides were used.

We found that both EGFR and P-cad were strongly associated with both CK5/6 and BRCA1 (ORs > 6.0 for all comparisons) but notably, cytoplasmic MDM2 staining was more strongly associated with some features of the basal phenotype (CK5/6, cyclin E, ORs >7.0, both P values <.0001) than with others (BRCA1 mutation, OR = 2.1, P = .18), implying a BRCA1-associated and a non-BRCA1-associated basal pathway. Furthermore, as noted for tumours expressing CK5/6 and/or carrying BRCA1 mutations, P-cad did not obey the size-nodes rule, in that large P-cad-positive breast cancers were not more likely to be node-positive than are small ones. In a multivariable Cox model including interaction terms for P-cad, tumour size and nodal status, P-cad was only associated with a poor prognosis in small, node-negative breast tumours, supporting the concept that basaloid breast cancers possess biological features that differentiate them from other types of breast cancer. This could be relevant both for the diagnosis and treatment of such cancers, and for BRCA1/2 testing prioritisation.

Cancer risk modifying effect of BARD1 SNPs in women carrying a BRCA1 mutation. *I. Coupier¹, D. Hughes², P-Y. Cousin¹, V. Gaborieau², D. Goldgar², O. Sinilnikova^{2,3}, D. Stoppa-Lyonnet¹, and. Groupe Genetique et Cancer⁴.* 1) Genetique Oncologique, Institut Curie, Paris, France; 2) Unite Epidemiologie Genetique, Centre de Recherche International sur le Cancer, Lyon, France; 3) Plate-forme mixte de genetique constitutionnelle des cancers frequents, HCL/CLB, Lyon, France; 4) B Bressac-de-Paillerets, A Chompret, YJ Bignon, JP Peyrat, J Fournier, C Lasset, S Giraud, D Muller, JP Fricker, A Hardouin, P Berthet, C Maugard, C Nogues, R Lidereau, M Longy, S Olschwang, C Toulas, R Guimbaud, S Mazoyer, G Lenoir.

Germline mutations in the BRCA1/2 genes strongly predispose heterozygous carriers to breast and ovarian cancer. The penetrance of these cancers is highly variable both between and within BRCA1/2 mutation carrier families. Such variations suggest that cancer risk associated with BRCA1/2 mutations can be modified by environmental and/or genetic factors. BRCA1 is a large protein with various interacting protein partners. Variants in the genes coding these proteins are thus good candidates to be modifier genetic factors. We have focused on SNPs of BARD1, whose protein interacts with the ring domain of BRCA1 and has an ubiquitin ligase activity. The study included 682 women recruited and identified as carriers of BRCA1 germline mutations. Of these 682 women, 110 have been diagnosed with ovarian cancer, 339 with breast cancer, 62 with both breast and ovarian cancers, and 171 women were breast and ovarian cancer free at the time of the last follow-up. Clinical characteristics of these women were available. Five BARD1 coding SNPs: P24S, T351T, R378S, H506H, V507M and, one non coding SNP, IVS5-14 A/G, were analysed. The SNPs were typed by TaqmanTM (ABI) using the ABI Prism 7700 Sequence Detection System or direct sequencing. The data were analysed by disease free survival analysis using a Cox proportional hazards model. A borderline significant association of the IVS5-14 A/G BARD1 variant with risk of ovarian cancer (HR = 1.9; CI = 1.20-2.68) was found. The identification of modifying factors on breast cancer risk in BRCA1 mutation carriers should help to refine individual risk estimation and might be helpful for clinical management.

Evaluation of RIZ in 1p36 as a Tumour Suppressor Gene for Pheochromocytomas and Abdominal

Paragangliomas. *J. Geli¹, B. Nord¹, T. Frisk¹, E. Edstrom Elder², T.J. Ekstrom³, T. Carling⁴, M. Backdahl², C.*

Larsson¹. 1) Center for Molecular Medicine, Karolinska Institute, Stockholm, Sweden; 2) Department of Surgery, Karolinska Hospital, Stockholm, Sweden; 3) Department of Clinical Neuroscience, Center for Molecular Medicine, Karolinska Institute, Stockholm Sweden; 4) Department of Surgery, Uppsala University Hospital, Uppsala, Sweden.

Pheochromocytomas and abdominal paragangliomas are rare catecholamine-producing tumours arising from neural crest derived chromaffin cells. Frequent deletions of several distinct regions on the short arm of chromosome 1, suggest their involvement in the tumorigenesis process. One potential candidate gene is the RIZ1 tumour suppressor in 1p36. A panel of 18 pheochromocytomas (10 benign, 4 malignant, 4 hereditary), and 11 abdominal paragangliomas (4 benign, 7 malignant) were characterised for somatic deletions and mRNA expression status of RIZ using loss of heterozygosity (LOH) analysis and real time quantitative PCR, respectively. Furthermore we have evaluated the RIZ1 promoter CpG island for methylation utilizing methylation specific PCR (MSP). Intragenic LOH at RIZ was detected in 10 of the 16 informative cases (63%) including 8 out of 12 pheochromocytomas (67%), and in 2 out of 4 paragangliomas (50%). RIZ1 mRNA appeared to be significantly underexpressed in the tumor samples compared to normal controls. We could not demonstrate RIZ1 promoter methylation in any of the samples indicating that promoter hypermethylation is unlikely to be the underlying cause to the frequent expressional silencing. The recurrent inactivation of the putative tumor suppressor RIZ1 suggests that this event may be a significant contributing factor to the tumour development in pheochromocytomas and abdominal paragangliomas.

Altered expression of cytokinesis gene, *SEPT9*, in breast cancer cells. *M.E. Gonzalez, L.M. Kalikin, E.M. Petty.* Int Med, Univ Mich, Ann Arbor, MI.

SEPT9 belongs to a highly conserved family of septins. It is essential for cell division and is implicated in leukemia. We cloned *SEPT9* from a region of allelic imbalance in breast and ovarian cancers. Its homologous to a murine proto-oncogene, *Sept9*, involved in lymphomas. *Sept9* is amplified and overexpressed in MMTV-pTg mammary adenocarcinomas. We hypothesized that altered expression of *SEPT9* variants might contribute to human breast cancer. To explore *SEPT9*'s role we examined endogenous expression of *SEPT9* variants in breast cancer cells and ectopic expression of isoforms in *in vitro* models. Semi-quantitative duplex PCR and Southern blot analysis demonstrated significant *SEPT9* amplification in 11 of 18 breast cancer cell lines (BCCs) compared to 0 of 4 immortalized normal human mammary epithelial cell lines (IHMECs) (p0.05). *SEPT9* was highly expressed in 60% of BCCs versus 25% of IHMECs by semi-quantitative duplex RT-PCR. High expression of *SEPT9v1*, *SEPT9v2*, and *SEPT9v4* was seen in BCCs compared to IHMECs. *SEPT9v3* and *SEPT9v5* were expressed in few BCCs and IHMECs. No sequence alterations were found to explain expression patterns. Using our polyclonal *SEPT9v1* Ab and a polyclonal *SEPT9* Ab that recognizes all isoforms (from W. Trimble), we analyzed immunoblots for protein expression and found high *SEPT9v1* expression in many BCCs compared to IHMECs (p0.025). *SEPT9*, *SEPT9v1*, and *SEPT9v2*-FLAG retroviral constructs were generated to express isoforms in epithelial cell culture models. Most notably, expression of pLNCX2-*SEPT9* and pLNCX2-*SEPT9v1*, but not vector controls, in RK3E and A2780 cells strikingly changed rounded epithelial phenotypes to elongated spindle shaped ones. Ectopic expression of *SEPT9v1*, not *SEPT9* or *SEPT9v2*, significantly increased invasiveness through a basement membrane matrix (Matrigel) compared to controls. Taken together, these findings suggest that preferential expression of alternatively spliced forms of *SEPT9* are important in breast cancer and specifically, that increased expression of *SEPT9v1* may contribute to malignant progression. Further studies are underway to explore this hypothesis and elucidate how altered expression of *SEPT9* variants arise and may impact cell division to drive oncogenesis.

Preliminary evidence for a prostate cancer susceptibility gene on chromosome 7p12.1 using unselected Ashkenazi

Jewish prostate cancer cases. *N. Hamel*^{1,4}, *K. Kotar*^{1,2,4}, *C.M. Greenwood*³, *W.D. Foulkes*^{1,2,4}. 1) Dept. Medicine, Montreal General Hosp, McGill Univ., Montreal, QC, Canada; 2) Cancer Prevention Centre, SMBD-Jewish General Hospital, McGill Univ., Montreal, QC, Canada; 3) Genetics and Genomic Biology, Hospital for Sick Children, Toronto, ON, Canada; 4) Program in Cancer Genetics, McGill Univ. Montreal, QC, Canada.

Prostate cancer is a common malignancy for which no clear susceptibility genes have been identified. Numerous genome-wide scans have indicated that prostate cancer is likely to be extremely heterogeneous genetically. Confining analysis to isolated populations with a limited number of founders can reduce locus heterogeneity and thereby increase detection power. Using this approach, a potential prostate cancer susceptibility locus was recently reported to exist on chromosome 7q11-21 by linkage analysis for 36 Ashkenazi Jewish (AJ) families.

Using the same rationale, we have been assembling a series of AJ prostate cancer cases unselected for family history. We are currently testing 160 cases and a group of 200 Israeli controls for 12 microsatellite markers on chromosome 7 for which significant linkage results were reported from fine mapping in the above analysis of AJ families. Preliminary results were obtained from a subset of cases and controls using the program PHASE (version 2.1) for 7 of the 12 markers (D7S1818, D7S1830, D7S502, D7S3046, D7S634, D7S2212, D7S820), spanning a 39-megabase interval. With 90 cases/90 controls, the overall permutation P value for all 7 markers was .00667. For p-arm markers D7S1818 and D7S1830 alone, and no exclusions for missing data, the P value was .0025 (400 permutations), whereas for the five q-arm markers D7S502 to D7S820, the P value was 0.36. When we compared the allele frequencies in cases and controls at each marker, only D7S1830 gave a significant result: χ^2 (6 DF) = 43.8, $P < .0001$. These preliminary findings, combined with reported HLOD data obtained from AJ families, suggest that a prostate cancer susceptibility gene may be located on 7p12.1.

TBX3 and its isoform, TBX3+2a, are Functionally Distinctive in Inhibition of Senescence and are Overexpressed in a Subset of Breast Cancer Cell Lines. *T. Huang¹, W. Fan¹, X. huang¹, C. Chen², J. Gray².* 1) Div Human Genetics, Univ California, Irvine, Irvine, CA; 2) Laboratory Medicine and Radiation Oncology, Cancer Genetics and Breast Oncology, UCSF Comprehensive Cancer Center, University of San Francisco, San Francisco, CA and Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA.

TBX3 is a transcription factor of the T-box gene family. Mutations of TBX3 cause Ulnar-Mammary syndrome (UMS, MIN 181450) in humans, an autosomal dominant disorder characterized by the absence or underdevelopment of the mammary glands and other congenital anomalies. Recently, it was found that TBX3 was able to immortalize mouse embryo fibroblast cells. In addition, TBX2, a homologue of TBX3, is very active in preventing senescence in rodent cells and was found to be amplified in some human breast cancers, suggesting TBX3 plays a role in breast cancer. This study examined the function of TBX3 and its isoforms, TBX3+2a. TBX3+2a differs from TBX3 in the DNA binding domain with an extra 20 amino acids produced by alternative splicing. We first examined the tissue expression and alternative splicing patterns of these two isoforms. We found that TBX3 and TBX3+2a are widely expressed in human and mouse and alternative splicing could be tissue-specific and species specific. Overexpression of TBX3 is able to immortalize mouse embryo fibroblast cells, while TBX3+2a shows an acceleration of senescence, a functional difference that may be explained by the fact that these two isoforms may have different downstream targets. TBX3, but not TBX3+2a, is able to bind to the previously identified T-box binding site in a gel shift assay. A subset of human breast cancer cell lines overexpresses TBX3. Our results indicate that TBX3 and TBX3+2a are functionally distinctive in inhibition of senescence of mouse embryo fibroblast cells and may play a role in breast cancer.

Comparison of Lung Adenocarcinoma and Squamous Cell Carcinoma Genomes. *W.W. Lockwood¹, C. Garnis¹, Y. Ge¹, S. Lam², C. MacAulay², W.L. Lam¹.* 1) Cancer Genetics, British Columbia Cancer Agency, Vancouver, B.C., Canada; 2) Cancer Imaging, British Columbia Cancer Agency, Vancouver, B.C., Canada.

Lung cancer is a worldwide problem and the most common cause of cancer-related deaths in North America. Non-small cell lung cancer (NSCLC) accounts for the majority of these cases, with squamous cell carcinoma (SqCC) and adenocarcinoma (AC) as the two major sub-types. SqCC develops more rapidly and arises near the central airways while AC originates from the epithelium of the lung periphery. The variation in progression and development of SqCC and AC may be due to underlying differences in genetic alterations between the two subtypes. Genetic abnormalities associated with each subtype have been reported. In this study, we compare SqCC and AC genomes using the newly developed sub-megabase resolution tiling-set (SMRT) array for whole genome comparative genomic hybridization (CGH) analysis. The SMRT array consists of 32,433 overlapping human bacterial artificial chromosome clones which span the entire human genome and allows detection of sub-megabase genetic changes. Using this technique, we generated profiles for 15 AC and 9 SqCC cell lines in order to distinguish the genetic features that characterize each subtype. While the two sample sets share recurrent regions of alteration, copy number changes specific to each subtype were also evident. SMRT array CGH enabled the detection of many novel aberrations which in turn define minimal regions of recurrent alterations that are potentially involved in tumorigenesis. Our results show that there are different genetic changes associated with SqCC and AC. This information could be used to tailor diagnosis and contribute to the understanding of the biology of the disease.

Frequent genetic alterations in both stromal and epithelial compartments of human epithelial ovarian cancer. A. Mannermaa^{1, 7}, H. Tuhkanen^{1, 2}, M. Anttila^{1, 3}, A. Kuronen⁴, M. Juhola⁴, R. Tammi⁵, M. Tammi⁵, V. Kataja², S. Helisalmi⁶, V.-M. Kosma¹. 1) Dept. of Clinical Pathology and Forensic Medicine, University of Kuopio and Kuopio University Hospital, Kuopio, Finland; 2) Dept. of Oncology, University of Kuopio and Kuopio University Hospital, Kuopio, Finland; 3) Dept. of Gynaecology and Obstetrics, University of Kuopio and Kuopio University Hospital, Kuopio, Finland; 4) Jyväskylä Central Hospital, Jyväskylä, Finland; 5) Dept. of Anatomy, University of Kuopio, Kuopio, Finland; 6) Dept. of Neurology and Neurosciences, University of Kuopio, Kuopio, Finland; 7) Dept. of Clinical Genetics, Oulu University Hospital and University of Oulu, Oulu, Finland.

We have shown that at 3p21.3 in ovarian cancer, allelic imbalance is found in 60-87% of the informative epithelial tumor cells of epithelial ovarian cancer and in adjacent stromal cells allelic imbalance is found at a frequency almost as high (52-80%)*. Our aim was to investigate the level and frequency of quantitative changes on cancer associated genes on both malignant epithelium and surrounding stroma in ovarian cancer by using MLPA technique (Multiplex Ligation dependent Probe Amplification). Altogether 110 genes covering all human chromosomes were analyzed from 24 epithelial and 13 stromal samples. We detected at least one altered gene in every tumor sample. No alterations from normal uterine tissue was found. The mean number of quantitative changes per tumor was 23.6 in epithelium and 10.8 in stroma. More specifically, there were in average 13.7 amplifications in epithelial cells and 6.6 in stromal cells. The mean number of deletions per tumor were 9.9 in epithelial cells and 4.2 in stromal cells. The most frequently altered gene was DCC in 18q21, which was lost in more than 60% of the samples. Although there was a clear trend for similar alterations in both epithelial and stromal parts of the tumor, we identified tumors where certain genes were altered only in stromal cells. Our study shows that stromal cells adjacent to epithelial ovarian cancer have undergone frequent genetic alterations and thus should be considered as an active part of the malignancy. *Tuhkanen et al. *Int J Cancer* (2004):109:247-52.

Detection of circulating tumour cells and lymph node micrometastasis in patients with bladder transitional cell carcinoma. *M. Marin-Aguilera*¹, *L. Mengual*¹, *M.J. Ribal*¹, *F. Algaba*¹, *E. Ars*¹, *R. Oliva*², *P.L. Fernández*², *H. Villavicencio*¹, *A. Alcaraz*¹. 1) Fundació Puigvert. Barcelona. Spain; 2) Hospital Clínic. Barcelona. Spain.

Transitional cell carcinoma (TCC) of the bladder is the fourth most common solid malignancy in males from industrialized countries and approximately 50% of patients with muscle-invasive disease treated by radical cystectomy die because of metastatic TCC. It remains unclear whether some tumour cell-derived mRNA transcripts detected in blood by PCR are appropriate molecular dissemination markers. We analyzed cytokeratin 20 (*CK20*) expression by RT-PCR as a method to detect circulating and residual tumour cells in blood, lymph nodes and bone marrow from patients with muscle invasive TCC. We studied 57 patients with invasive TCC treated with radical cystectomy and 9 patients with non-invasive TCC. None of them presented metastasis in preoperative clinical staging. Twenty-two blood samples, 5 bone marrow samples and 5 lymph node samples from patients with no history of TCC served as negative controls. Four blood samples from patients with known metastatic disease were the positive controls.

None of the negative controls expressed *CK20*. In bone marrow samples, 19% of patients showed *CK20* expression. In peripheral blood 30% of patients expressed *CK20* and only one of them had a non-invasive TCC. Three of the 4 positive controls showed *CK20* expression in peripheral blood. Lymph node pathological status was positive in 42% of patients with invasive TCC and all of them showed expression of *CK20* except for one case. Of the pathologically negative lymph nodes, *CK20* was detected in 46,6%.

CK20 RT-PCR assay appears to be a highly sensitive and specific method for detecting circulating tumour cells and residual disease in lymph node from patients with invasive bladder cancer. However, it is necessary to further evaluate *CK20* transcripts as a molecular tumour marker in blood performing the follow-up of the patients to assess the clinical significance of the disseminated cells.

Partially degraded RNA from bladder washings is suitable to study gene expression patterns of bladder cancer using DNA microarrays. *L. Mengual¹, M. Buset¹, E. Ars¹, M.J. Ribal¹, F. Algaba¹, Y. Arce¹, L. Sumoy², J.J. Lozano², B. Miñana², M. Marín-Aguilera¹, M. Fernández¹, L. Sedano¹, H. Villavicencio¹, A. Alcaraz¹.* 1) Molecular Biology Lab., Fundació Puigvert; 2) Microarrays Lab., Centre de Regulació Genòmica. Barcelona. Spain.

The determination of gene expression profiles in bladder cancer using DNA microarrays is usually performed with RNA isolated from tumour samples. The study by microarray technology of bladder fluids in contact with the tumour, such as urine or bladder washings, could be of interest because they may be obtained with minimally invasive or non-invasive procedures. However, the isolation of high quality RNA from such samples constitutes a frequent problem. The aims of this study were to determine if gene expression profiles were modified by RNA degradation and to correlate gene expression profiles between exfoliated cells from bladder washings and bladder cells tumours. Total bladder tumour and bladder washing RNA from the same patient was heat shocked to obtain 4 degradation levels. We amplified these RNAs by using the T3N9 method suitable for degraded RNAs. Finally, we hybridised cDNA microarrays with probes generated with this amplified RNA. After the microarray data was filtered and normalized, the top and bottom 5% (426) of ranked clones were compared in all samples. Our results showed that even with the badly degraded RNA we detected most of the differentially expressed genes found in intact samples (mean 63% in tissue samples and mean 79% in bladder washings). Furthermore, we could detect a high percentage of differentially expressed genes (mean 54%) in common between bladder washings and tumours. Expression differences for 10 genes were independently confirmed by real-time PCR. Our results suggest that gene expression profiles obtained from partially degraded RNA samples with conserved ribosomal bands have a high degree of similarity compared to intact samples when using the T3N9 amplification method. Also, we show that there is a good correlation between gene expression patterns in bladder washings and tumours, making the bladder washing a suitable sample to study gene expression profiles of bladder cancer.

BRCA unknown variants impact on splicing. *V. Moncoutier¹, D. Michaux¹, S. Pagès Berhouet¹, C. Dehainault¹, A. Laugé¹, I. Coupier¹, M. Gauthier Villars¹, D. Stoppa Lyonnet^{1, 2}, C. Houdayer¹.* 1) Genetics; 2) Inserm U509, Institut Curie, Paris, France.

Breast cancer is a leading cause of cancer deaths among women, and it is estimated that 5%-10% of cases are caused by inherited mutations in the breast cancer susceptibility genes BRCA1, 2 (BRCA). Most of the deleterious BRCA mutations leads to a truncated protein. Surprisingly, splicing mutations would account for a mere 5% of BRCA mutational spectrum, as opposed to NF1 or ATM that exhibit 50% of splicing mutations. However, BRCA molecular analysis in at-risk women shows that nearly half of BRCA sequence variations are of uncertain clinical significance, i.e. the so-called Unknown Variants (UV). Therefore, we embarked upon systematic characterization of UV at the RNA level and report here our preliminary results on 20 BRCA UVs, located outside the AG/GT canonical sites. We used a combination of web-based tools (Splice Site Finder, Splice Site Prediction, GeneSplicer, MaxEntScan, ESE Finder and Rescue ESE) and RT PCR analyses. Total RNA was extracted from lymphoblastoid cell lines. For each patient, one cell line was treated with puromycin and one cell line was left untreated to optimize the detection of unstable transcripts. Following electrophoresis, all RT PCR fragments were gel-cut and sequenced. We also looked for the presence of polymorphisms at the cDNA level to detect a possible hemizygoty due to an unstable mutant transcript. To study allelic imbalance, an allele-specific RT PCR with Locked Nucleic Acid primers is under development. We found that 8 of the 20 UVs were actually leading to splice alterations, that ranged from exon skipping to more complex defects such as intron retention or a combination of both defects. Our preliminary results suggest that a putative impact of UVs on splicing should be systematically looked for. This study will be enlarged to 50 unselected UVs identified during the course of BRCA molecular diagnosis, with the aim of defining a strategy for a routine-based diagnosis, possibly by a combination of first line in silico analysis followed by RT PCR analysis.

Genomic Alterations Identified by cDNA Microarray Analysis in Cervical Cancer: MMP Genes are Targets of 11q22 Amplicon. *G. Narayan*¹, *V. Bourdon*², *S. Chaganti*², *H. Arias-Pulido*⁴, *S. Nandula*¹, *P.H. Rao*³, *A. Schneider*⁵, *M. Durst*⁵, *L. Gissmann*⁶, *M. Mansukhani*¹, *R.S. Chaganti*², *V.V. Murty*¹. 1) Dept Pathology, Columbia Univ, New York, NY; 2) Memorial Sloan-Kettering Cancer Center, New York, NY; 3) Baylor College of Medicine, Houston, TX; 4) University of New Mexico, Albuquerque, NM; 5) Friedrich Schiller Univ, Jena, Germany; 6) Deutsches Krebsforschungszentrum, Angewandte Tumorstudiologie, Im Neuenheimer Feld 242, Heidelberg, Germany.

Cervical cancer (CC) is a leading cause of cancer-related deaths in women worldwide. CC displays a myriad of cytogenetic abnormalities including chromosomal and gene amplifications, mutations in certain genes as well as epigenetic changes. Despite this, the molecular basis of CC development is still poorly understood. Here, we analyzed 29 CC specimens by microarray comparative genomic hybridization on an array containing 9,206 cDNA clones to identify gene dosage alterations. This analysis identified DNA copy number increase in 445 clones and deletions in 121 clones in 3 or more tumors. The chromosomes X, 1, 5, 3, 19, and 20 were most commonly showed overrepresented genes. A number of these amplified genes associated with distinct biological processes in tumor formation. This analysis also further identified 17 potential amplicons (4 on X chromosome, 3 on chromosome 1, one each at 3q27-q29, 5p13, 5q31, 8q24, 11q22-q23, 14q32, 17q21-q22, 19p13, 19q13.3, and 20q13.2). The deleted genes were commonly mapped to the chromosome regions 4q13.3, 2q33-q27, and 13q14.1 suggesting that these sites harbor candidate tumor suppressor genes. The deleted genes include tumor suppressors (FHIT and ZDHHC2), apoptosis (CASP1), transcription factors, immune response, cell-cell interaction, and DNA repair genes. A number of the amplified and deleted genes were further validated by fluorescence in situ hybridization, Southern and/or northern blot analysis, and RT-PCR. Characterization of an 800 kb size amplicon mapped to 11q22 identified matrix metalloproteinases MMP1, MMP12, and MMP13 as critical target genes of this amplicon. Thus, we have identified number critical genes of relevance to CC development.

Effect of NF2 missense mutations on nuclear transport. *B. Schmucker, M. Kressel.* Institute of Anatomy, University of Erlangen, Germany.

Tumor suppressor proteins are regulated by a number of different strategies to prevent abnormal cellular growth. Many of them shuttle between the nucleus and the cytoplasm thus regulating their function. Alteration of nuclear transport, e.g. mutations in the nuclear localisation sequences (BRCA1 gene), truncation of the nuclear export signal (APC gene) or defect in nuclear-cytoplasmic targeting (p53) lead to initiation or progression of cancer. Previously, we identified a C-terminal nuclear export signal (NES) in the Neurofibromatosis 2 protein (NF2) and demonstrated that NF2 shuttles between the nucleus and the cytoplasm. To investigate the impact of frameshift mutations (most common genetic alteration in NF2 patients) on nuclear transport, we created a full-length NF2 construct terminated at residue 535 (L535X). Despite truncation 5 of the NES, the construct localized at the plasma membrane and did not enter the nucleus. Additionally, missense mutations which were found in NF2 tumors in combination with loss of the remaining NF2 allele, were expressed and analyzed for nuclear transport: L46R, L54P, F62S, L64P, K79E, E392V, K533T, L535P. However, only a slightly increased nuclear localization was observed compared to wt protein with a relatively stronger effect of mutations mapping to exon 2. Recently, protein 4.1 was shown to exhibit a NES in a region highly homologous to NF2 exon 2. Mutagenesis of this homologous domain in NF2 wt-protein (L54P-L56Q; L54A-L55A-L56A) did not alter the nuclear localization. Any putative NES within exon 2 was additionally excluded by an Rev-nuclear export assay (as developed by Henderson et Eleftheriou 2000). Furthermore, we analyzed the NF2 BlueBox mutation, a 7 amino acid stretch in the FERM domain of NF2, known to increase proliferation and induce malignant transformation. However, no effect of this mutation on nuclear uptake was observed. Since phosphorylation of serine 518 is suggested to convert Merlin from antiproliferative to growth-permissive state, we investigated S518D and S518A mutations, mimicking constitutively phosphorylated and dephosphorylated NF2, respectively. No alteration of nuclear transport was detected, indicating that nuclear entry of NF2 is not dependent on phosphorylation.

Premature senescence identified as a primary tumor-protective response of breast carcinoma cells following

inducible oncogenic ERBB2-signalling. *C. Spangenberg¹, T. Trost¹, E. Lausch¹, S. Schmitt², S. Fees¹, J.G.*

Hengstler³, E.O. Bockamp², D. Prawitt¹, B. Zabel¹. 1) Children's Hospital, University of Mainz, Germany; 2) Institute of Toxicology, University of Mainz, Germany; 3) Institute of Toxicology, University of Leipzig, Germany.

ERBB2 is a member of the epidermal growth factor receptor family of receptor tyrosine kinases. It is causally implicated in the development of a subset of breast cancer and other epithelial malignancies. Enhanced ERBB2 expression leading to aberrant signalling can frequently be attributed to genomic amplification of the *ERBB2*-locus on chromosome 17q21. In the work presented we apply a tetracycline-controlled expression system to inducibly express oncogenic ERBB2 (NeuT) in MCF-7 breast carcinoma cells. Expression of NeuT results in prominent phenotypic alterations in several independent MCF7/Tet-NeuT clones 16-48h after addition of tetracycline to the culture medium. Interestingly, oncogenic ERBB2 signalling in these cells does not lead to a mitogenic response but instead induces features of premature senescence, like cell cycle arrest and expression of senescence associated beta-galactosidase. Further molecular analysis provide direct evidence that upregulation of the cyclin dependent kinase inhibitor P21 via the P38 MAPK pathway is necessary to elicit premature senescence in response to oncogenic ERBB2 signalling. Premature senescence represents a tumor-protective program that has previously been shown to be induced by overexpression of other oncogenes, like activated forms of RAS or RAF. Taken together our results indicate that ERBB2-driven mammary tumorigenesis might involve additional hits functionally inactivating the cellular premature senescence response. These lesions may center around the P21 molecule or target downstream effectors of senescence.

Transcriptome analysis of a candidate ovarian cancer (OC) tumor suppressor gene region at 17q25. *P. Tonin¹, K. Dewar^{1,2}, V. Forgetta², D. Provencher³, A.-M. Mes-Masson³, N. Presneau¹.* 1) Dept Human Genetics, McGill U., Montreal, PQ; 2) McGill U. and Genome Quebec Innovation Centre, Montreal, PQ; 3) CR-CHUM (Hopital Notre Dame)/Institut du cancer de Montreal, Montreal, PQ.

Loss of heterozygosity (LOH) analysis was performed in OC to further characterize a previously identified candidate tumor suppressor gene region encompassing D17S801 at 17q25.1. LOH of at least 1 informative marker was observed for 100 of 140 OC samples in an analysis of 6 polymorphic markers (cen-D17S1839-D17S785-D17S1817-D17S801-D17S751-D17S722-tel). The combined analysis revealed a 453 Kb minimal region of deletion (MRD) bounded by D17S1817 and D17S751. Human and mouse genome assemblies were used to resolve marker inconsistencies of the D17S1839-D17S722 interval and identify candidates for expression analysis. The region contains 32 known and strongly predicted genes, 9 of which overlap the MRD. The two reference genomic sequences share nearly identical gene structures. The organization of the region is highly collinear. The 17q25 region does not show any large internal duplications. A 1.5 Kb inverted duplicated sequence of 87% nucleotide identity was observed in a 13 kb region surrounding D17S801. Transcriptome analysis by Affymetrix GeneChip and RT-PCR methods of 3 well characterized OC cell lines and primary cultures of normal ovarian surface epithelial cells was performed with 32 candidates spanning the D17S1839-D17S722 interval. RT-PCR analysis of 8 known or strongly predicted genes residing in the MRD in 10 OC samples, that exhibited LOH of the MRD, identified FLJ22341, as a strong candidate. Differential expression by RT-PCR of FLJ22341 was also observed in 1 of 3 EOC cell lines but not by microarray expression analysis. The proximal repeat sequence of D17S801 occurs 8 Kb upstream of the putative promoter region of FLJ22341. RT-PCR analysis of the OC samples and cell lines also identified DKFZP434P0316, a gene that maps proximal to the MRD, as a candidate. While Affymetrix technology was useful for initially eliminating less promising candidates, subsequent RT-PCR analysis of well-characterized OC samples was essential to prioritize candidates for further study.

The influence of the polymorphisms of the enzymes that regulate the homeostasis of folic acid on the plasmatic levels of Homocysteine in children with acute lymphoblastic leukemia. *F. Garcia Bournissen¹, M. Primeau¹, A. Moghrabi¹, M. Krajinovic^{1, 2}.* 1) Service d'Hematologie-Oncologie, Centre de Cancerologie Charles-Bruneau et Centre de Recherche, Hopital Sainte-Justine; 2) Departement de Pediatrie, Universite de Montreal.

INTRODUCTION: Acute lymphoblastic leukemia (ALL) is the most frequent cancer of childhood, with a current cure rate of 80%. However, ALL treatment can be associated with both resistance and toxicity. It has been suggested that intrathecal methotrexate (MTX) could contribute to a decline in neurocognitive functioning. Homocysteinemia, due to its pathogenic effect on vascular endothelium, is thought to play a role in the development of this toxicity. Several enzymes are essential to maintain the homocysteine (Hcy) levels. Their different functional forms, associated with common genetic polymorphisms may act as predisposing factors of MTX-related neurotoxicity. **MATERIAL AND METHODS:** Sixty children treated with DFCI-2000 protocols were included in the prospective study conducted at the Ste-Justine Hospital (Montreal) in the 2001-03 period. Hcy levels were measured by fluorescence polarization immunoassay in plasma samples obtained at diagnosis and 24hs following a high MTX dose. The polymorphisms in genes relevant for Hcy homeostasis were analyzed including methionine synthase (MTR), methionine synthase reductase (MTRR), methylene tetrahydrofolate reductase (MTHFR), cystathionine beta-synthase (CBS) and endothelial nitric oxide synthase (eNOS). MTHFR C677T and A1298T, MTR A2756G, eNOS T-786C and G849T, and MTRR A66G were studied by PCR-ASO. The presence of a CBS 68bp insertion was detected by PCR followed by gel electrophoresis. Statistical analyses were carried out by repeated measures ANOVA. **RESULTS:** An increase in Hcy levels following MTX administration was observed ($p < 0.001$) as expected. Regarding genotype-Hcy relationship (within subject effect), the presence of the TT genotype of eNOS T-786C polymorphism was significantly associated with the increase of Hcy levels from the baseline values ($p = 0.01$). **CONCLUSION:** The potential predictive role of eNOS TT-786 homozygosity should be confirmed in clinical setting.

Identification of single nucleotide polymorphisms (SNPs) associated with adverse effects (AEs) of gefitinib

(Iressa). *M. Isomura*¹, *M. Fukuoka*², *S. Sone*³, *M. Matsuura*¹, *T. Noda*¹, *T. Muto*¹, *Y. Miki*¹. 1) Genome Center, Cancer Inst., Toshima, Tokyo, Japan; 2) Dep. Medical Oncology, Kinki Univ., Osaka, Japan; 3) Dep. Internal Medicine, Tokushima Univ., Tokushima, Japan.

The efficacy of gefitinib (Iressa) in NSCLC has been well demonstrated in clinical studies. However, AEs of gefitinib treatment were also observed. A method for predicting AEs may be a useful tool. In order to predict AEs of gefitinib, we conducted large-scale SNP typing analyses; 42 patients (pts) with NSCLC were enrolled. All pts received oral gefitinib 250 mg/day for 16 weeks. AEs were evaluated every 7 days (NCI-CTC grading). Gefitinib concentration in peripheral blood was measured on day 28. To identify SNPs associated with AEs, DNA was isolated from peripheral blood and genotypes of 3,717 SNPs over 512 genes were determined by Invader assay. Association between SNPs and AEs was examined using Fishers exact test. During treatment, 23/42 pts exhibited diarrhea. In 22/23 pts diarrhea was mild and manageable (CTC grade 2), and less severe than with traditional chemotherapy. Genotyping analyses showed a strong association between diarrhea and 7 SNPs located within SLC22A4 gene ($p=0.000025$, odds ratio: 22.3). Among those, 3 SNPs were located within the putative regulatory region of SLC22A4 and the rest were within the intronic region, suggesting these polymorphisms may alter gene expression. The SNPs belonged to 1 of 3 major haplotypes observed at the locus, suggesting a specific haplotype may harbor genetic alteration responsible for developing diarrhea. For other AEs the number of affected pts was too small to analyze for an association with SNPs. Two SNPs on SLC22A7 gene were strongly associated with gefitinib blood concentration ($p=0.00056$, odds ratio: 15.1). No correlation was seen between appearance of diarrhea and high gefitinib blood concentration. By using these SNPs the appearance of diarrhea on gefitinib could be precisely predicted. Both genes belonged to same gene family, suggesting this family is involved in pharmacokinetics and/or pharmacodynamics of gefitinib. Iressa is a trademark of the AstraZeneca group of companies.

ESOPHAGEAL SQUAMOUS CELL CARCINOMA AND IL1B GENE PROMOTER REGION

POLYMORPHISMS. *K. Azim, K. Brophy, R. McManus, D. Kelleher, J.V. Reynolds.* Institute of Molecular Med, Trinity College Dublin, Dublin, Ireland.

Purpose: To estimate the distribution of IL-1B -511 C-T biallelic single nucleotide polymorphism (SNP) in patients with cancer of the esophagus and a control population of Irish origin. **Methods:** In a retrospective case-control study design we selected 222 esophageal cancer cases and 228 controls. DNA was extracted and genotyping was performed using PCR-RFLP and TAQMAN chemistry. The study had local Ethical Committee approval and informed consent was obtained from all study subjects. **Results:** The distribution of CC homozygotes (51.8% in cases, 39.9% in controls) and CT heterozygotes (36.5% in cases, 48.2% in controls) was significantly different in the two study groups ($p = 0.028$). However, this difference was reflected only in squamous cell cancer patients ($n=88$, $p = 0.001$), CC homozygotes (63.6% in cases, 39.9% in controls) and CT heterozygotes (26.1% in cases, 48.2% in controls). There was no difference in the distribution of genotypes in adenocarcinoma cases and controls ($n = 128$, $p = 0.712$). Genotypes were not associated with stage of disease but larger tumor sizes were observed in patients carrying C allele compared to patients carrying T allele (Mean tumor size; CC = 4.02cm, CT = 3.44 cm, TT = 2.76 cm. $p = 0.017$). **Conclusion:** The distribution of histological types of esophageal cancer is geographical as well as race and gender related. Although the last three decades have witnessed an increase in incidence of adenocarcinomas in Caucasian population for unknown reasons, squamous cell carcinoma of the esophagus has been related to smoking, diet, and alcohol consumption. Polymorphisms in IL 1B gene cluster have been implicated in many diseases including cancers. This study shows individuals carrying CC genotype of IL 1B gene may be at higher risk of developing squamous cell esophageal cancer in some way that needs further investigation.

A Study of the Association Between the Glycine N-Methyltransferase (GNMT) Gene Polymorphism and the Susceptibility to Prostate Cancer. *Y.-M.A. Chen¹, Y.-C. Huang¹, M.-Y. Chung², Y.-H. Chang³, J.-S.W. Huang^{3,4}, M.-T.D. Ho⁵, C.-C. Pan⁵, T.-L. Wu⁶.* 1) Institute of Public Health, National Yang-Ming University, Taipei, Taiwan; 2) Department of Medical Research and Education, Taipei Veterans General Hospital, Taipei, Taiwan; 3) Department of Surgery, Taipei Veterans General Hospital, Taipei, Taiwan; 4) Faculty of Medicine, National Yang-Ming University, Taipei, Taiwan; 5) Department of Pathology and Laboratory Medicine, Taipei Veterans General Hospital, Taipei, Taiwan; 6) Department of Surgery, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan.

Glycine N-methyltransferase (GNMT), a protein with multiple functions. We have previously reported on diminished GNMT expression levels in both human hepatocellular carcinoma (HCC) cell lines and tumorous tissues. Since GNMT only expresses in prostate, liver, kidney and pancreas. Here we were a) investigate the GNMT expression levels in prostate cell lines and tissues and b) study the association between the GNMT polymorphism and the susceptibility to prostate cancer (PCa). The results of Western blot showed that none of 8 PCa and 2 normal prostate cell lines expressed GNMT while its mRNA can be detected by RT-PCR in all the PCa cell lines. Immunohistochemical staining with GNMT monoclonal antibody 14-1 showed that GNMT was undetectable in 75% (33/44) of the tumor adjacent tissue and 84% (38/44) of the tumor tissues from PCa patients. The case-control study which comprised 163 PCa patients and 226 age-matched controls of GNMT SNP1 genotype showed that the odds ratio (OR) of developing PCa in persons with C/C genotype was 1.40 (95% CI= 0.88-2.23) when we used both C/T and T/T genotypes as a reference. In addition, 4 of 20 (20.0%) and 6 of 21 (28.6%) PCa tumor tissues had LOH of the INS/DEL and STRP1 genotype. The expression of GNMT was found to be downregulated in PCa cell lines and tissues. 20.0-28.6% of PCa tumor tissues had LOH of the genetic markers of GNMT. The C-allele of SNP1 maybe associated with increased PCa risk. Therefore, GNMT is a tumor susceptibility gene for PCa.

Large scale, high throughput genotyping for testis and bladder tumour candidate genes. *M.J.H. Coenen¹, F. de Vegt², K.K. Aben², M.M.V.A.P. Schijvenaars¹, C. de Kovel¹, L.A.L.M. Kiemeny², B. Franke¹.* 1) Human Genetics, UMC Nijmegen, Nijmegen, Netherlands; 2) Department of Epidemiology and Biostatistics, UMC Nijmegen, Nijmegen, Netherlands.

The effect of environmental/life-style factors on the etiology of tumours is modified by a persons genetic susceptibility. This genetic susceptibility has been studied in small-scale studies mainly concerning genes involved in carcinogen metabolism. Now that high throughput technologies have become available we want to perform large scale genotyping to identify genetic variations (in combination with life-style factors) that increase the risk for testis and bladder tumours using a SNP microarray approach. To identify genetic markers in combination with life-style factors a case/control study will be performed with 700 bladder cancer patients; 500 testicular germ cell tumour patients and age/sex matched controls. All cases and controls will be genotyped using a microarray containing genetic variants (single nucleotide polymorphisms (SNPs)) of about 150 genes known to be involved in cancer related pathways (for instance genes active in carcinogen metabolism, DNA repair or cell cycle control). In each of these 150 genes three SNPs have been selected using information from publicly available databases and the Celera SNP database. We have preferentially selected functional SNPs which are known or suspected to change gene product function or regulation. Genotyping will be performed by SNP microarray analysis. This enables us to analyse 400-500 SNPs simultaneously. The SNPs are genotyped by a four-colour microarray-based mini-sequencing assay. Cyclic mini-sequencing is performed with 5 tagged primers followed by capture of the products on microarrays by hybridisation to complementary tag oligonucleotides. Finally statistical analysis will be utilised to identify individual SNPs as well as patterns of SNPs which are associated with testis and bladder tumours. We hypothesise that the combination of large scale genotyping with information regarding environmental risk factors will give a better insight into the pathogenic mechanisms of testis and bladder tumours.

The XRCC2 R188H variant has a significant effect on survival from breast cancer. A. Cox¹, S. Rafii¹, G. MacPherson¹, S. Balasubramanian², M. Meuth¹. 1) Inst Cancer Studies, Med Sch, Univ Sheffield, Sheffield, UK; 2) Academic Surgical Oncology Unit, Med Sch, Univ Sheffield, Sheffield, UK.

The protein products of the breast cancer genes, BRCA1 and BRCA2, are known to be involved in the repair of double strand breaks (DSB) in DNA. We and others have shown that common variants of genes of the DSB repair pathway can act as low penetrance breast cancer susceptibility alleles. Two large studies have shown that the rare allele of XRCC2 R188H variant is associated with breast cancer (with combined odds ratio for the rare homozygote of 2.11 (95% confidence interval (CI) 1.06, 4.20). The arginine residue at position 188 is necessary for efficient repair of DSB in vitro. DSB repair genes are also strong candidates to affect treatment outcome and survival. Therefore we tested the hypothesis that coding germ-line variants in DSB repair genes were associated with survival from breast cancer. Blood DNA samples were obtained from 1008 women with histopathologically confirmed breast cancer attending surgical clinics at the Royal Hallamshire Hospital in Sheffield UK, between November 1998 and June 2002. Extensive demographic, epidemiological and pathological data were also recorded. Mortality data was obtained from Trent Cancer Registry in April 2004. We genotyped this cohort for coding SNPs in a panel of DSB repair genes using the TaqmanTM (Applied Biosystems) 5 nuclease PCR assay. Survival in different genotype groups was compared using Cox regression analysis, controlling for known prognostic factors. Coding SNPs in the genes ATM, BRCA2, LIG4, XRCC3, and RAD51 had no significant effect on survival in this study. However, women homozygous for the rare allele of the XRCC2 R188H SNP had poorer survival compared to women homozygous for the common allele (hazard ratio (HR) 2.54, (95% CI 1.54, 4.21). There was no significant effect of heterozygosity of this allele (HR 1.30, 95%CI 0.78, 2.17). While this result requires confirmation, the association of the H allele with both breast cancer susceptibility and poor survival suggests that DNA repair capacity may be an important factor determining outcome in breast cancer.

IFN- Intron-1 polymorphism in sporadic breast cancer patients and an in-vitro expression profile. *A. Dhir, A. Saha, A. Ranjan, V. Gupta, N.K. Bairwa, R. Bamezai.* NCAHG, SLS, Jawaharlal Nehru University, New Delhi, Delhi, India.

The first intervening sequence (Intron-1) of interferon gamma (IFN-) gene is comprised of a tissue non-specific enhancer element. Its influence on the expression of interferon has not been very clear although previous reports have suggested an increase in the interferon level associated with an allele of 12 repeats of CA, (CA)₁₂, in this region of the gene. Here we have attempted to study the prevalence of (CA)_n polymorphism in the intron-1 of IFN- gene in a case control study of sporadic breast cancer patients to find out the difference in the rate of prevalence at germline and somatic level in this region of the gene, considered to modulate the expression of this critical cytokine, influencing tumor growth and progression. The (CA)₁₂ allele was observed in 50% of patients in comparison to 15% controls (p=0.0001) as a germline pattern. Further, 9/54 (17%) tumor samples depicted an allelic variation and the presence of mutations in the 5'flanking region (+874, as per HUGO nomenclature) of the (CA)_n repeat as a somatic event. An analysis of the influence of (CA)₁₂ repeat on the expression in a reporter assay suggested this allelic form to be a downregulator of the expression when compared to other allelic forms. The observation of presence of (CA)₁₂ repeat in the intron-1 of IFN- gene in a significant percentage of patients when compared to normals and the association of this allelic form with downregulation of IFN- , points clearly to the downregulation of the Th1 surveillance of immune response in such individuals. The present study provides an initial evidence of how a genotype background of one of the critical cytokines in immune response could probably influence the nature of tumor growth and proliferation in sporadic breast tumors. This would be in addition to a variety of factors, which play their respective roles in containing the tumor growth and their failure results into an uncontrolled tumor growth and invasion in other sites of the body.

Cytochrome P4502D6 G1846T/A polymorphism is associated with susceptibility to breast cancer in Mexican patients. *M.P. Gallegos Sr¹, L. Arnaud-López¹, L.E. Figuera², I.P. Dávalos-Rodríguez², G.M. Zúñiga-González¹.* 1) Medicina Molec, CIBO IMSS, Guadalajara Jalisco, Mexico; 2) Genética, CIBO, IMSS, Guadalajara, Jalisco, Méx.

Breast cancer is the most frequent malignancy among women. Given genetic factors such as BRCA1 and BRCA2 as well as reproductive history constitute only 30% of the cause, environmental elements could play a significant role in the development of breast cancer. Likewise, the relevant enzymes involved in the biotransformation of xenobiotics (from tobacco smoke, diet or other environmental sources) might play a role in breast carcinogenesis. Some individuals with modified ability to metabolize these carcinogens could have a different risk for breast cancer. We investigated the role of cytochromes P-450 CYP2D6 G1846T/A polymorphism in breast carcinogenesis. A case-control study was conducted on 104 women with breast carcinoma and 100 healthy controls. The prevalence of genotype G1846T/A in normal controls and cancer patients was studied using the PCR method. In the study groups, 48% were found to carry G1846/G1846 in the control and 19% in the patients, while 38% carried G1846/T/A1846 in controls and 48% in patients group and 14% were T/A1846/T/A1846 in control and 33% in patients group. The CYP2D6 G1846T/A polymorphism low acetylator was found to be a significant risk determinant of breast carcinoma (OR = 3.9, 95% CI 1.8-8.3). These data suggest that CYP2D6 G1846T/A polymorphism could play a role in the susceptibility to breast carcinoma in Mexican population.

MDR1 C3435T polymorphism as a risk factor for childhood acute lymphoblastic leukemia. *F. Rivas Jr.^{1,2}, M. Ortiz^{2,3}, K. Rodarte^{2,3}, R. Ortiz^{2,4}, J.C. Aguilar⁵, M.A. Esparza⁵, B. Lopez⁵, N. Olivares⁶, F. Rivas².* 1) School of Medicine, Universidad de Colima, Colima; 2) Western Biomedical Research Center, Instituto Mexicano del Seguro Social; 3) Graduate Studies Program in Human Genetics, Universidad de Guadalajara; 4) Graduate Studies Program in Immunology, Universidad de Guadalajara; 5) Servicio de Hematología, Hospital de Especialidades CMNO-IMSS; 6) Hospital General de Occidente, Secretaria de Salud Jalisco, Guadalajara, Jalisco, Mexico.

Xenobiotic and drug transporter molecules are attractive candidate risk factors for cancer susceptibility. One of such molecules is the ATP binding cassette family P-glycoprotein (P-gp), coded by the MDR1 gene at 7q21.1. The C3435T SNP at this gene correlates with P-gp expression and activity in different tissues. The T allele relates with low P-gp, probably due to linkage with a yet unknown mutant. It has been proposed as a risk factor for the occurrence of childhood acute lymphoblastic leukemia (ALL) and other malignancies, while the C allele (increased P-gp) would be associated with a worse outcome. In this study allele and genotype frequencies from 52 ALL children were compared with those from 82 healthy unrelated Mestizo individuals living in Guadalajara Mexico. MDR1 C3435T was typed by PCR-RFLP (*MboI*) in DNA samples extracted from peripheral blood. Allele frequencies were 101/164 (62%) C and 63/164 (38%) T in controls; and 56/104 (54%) C and 48/104 (46%) T in the ALL patients ($p=0.25$). Genotype counts were 32 (39%) CC, 37 (45%) CT, and 13 (16%) TT in controls. These proportions do not deviate from Hardy-Weinberg Expectations. Genotypes in cases: 11 (21%) CC, 34 (65%) CT, and 7 (14%) TT did not differ from those in controls ($p\sim 0.06$). The T phenotype (CT+TT), however, was significantly higher ($p=0.034$) in ALL children than in controls, with an OR=2.39 ($CI_{95}=1.00-5.75$). Besides describing population genetic estimates for this SNP for the first time in Mexicans, the present results suggest that the MDR1 C3435T SNP influences an individual's susceptibility to ALL, likely through differential P-gp expression.

Genetic polymorphism of Glutathione S-Transferase genes (GSTM1, GSTT1, and GSTP1) and susceptibility to prostate cancer in northern India. *D.S.L. Srivastava¹, A. Mandhani¹, B. Mittal², R.D. Mittal¹.* 1) Department of Urology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow-226014, Uttar Pradesh, India; 2) Department of Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow-226014, Uttar Pradesh, India.

Glutathione-S-transferases (GSTs) are active in the detoxification of wide variety of endogenous or exogenous carcinogens. The genetic polymorphism of GSTM1, GSTT1 and GSTP1 genes has been studied earlier to evaluate the relative risk of various cancers. In the present study, we examined the association of the GST gene polymorphisms with sporadic prostate cancer patients. Our study included 127 individuals with histologically confirmed prostate cancer and 144 age-matched controls from North India. DNA was isolated from peripheral blood by standard phenol chloroform method. GSTT1 and GSTM1 null genotypes were identified by multiplex PCR and GSTP1 313 A/G polymorphism was determined by PCR/RFLP. We observed significant association in null alleles of the GSTM1 (OR=2.239, 95% CI= 1.37-3.65, P = 0.001) and GSTT1 (OR=1.891, 95% CI= 1.089-3.282, P = 0.026) with prostate cancer risk; as well as 313 G alleles of the GSTP1 gene (OR = 2.48, 95% CI= 1.51-4.08, P= 0.000). The combined analysis of the above three genotypes showed further increase in the risk of prostate cancer. (OR = 7.23, 95% CI = 2.42-22.63, P= 0.000). Our study demonstrated that GSTP1 313 G polymorphism, and null alleles of GSTM1 and GSTT1 are strong predisposing risk factors for sporadic prostate cancer in North India.

Molecular studies on frequency of single nucleotide polymorphism of cyclin D1 gene (G870A) in Iranian patients with gastric cancer. *M. Vosoughi¹, F. Sharifpanah¹, F. Tirgari², N. Abedin¹, M. Karimi³, L. Emadian¹, A.*

Mohagheghi³, B. Noorinayer¹, M.R. Zali¹. 1) Cancer Genetics Department, Research Center for Gastroenterology and Liver Diseases (RCGLD), Tehran, Iran; 2) Cancer Institute of Tehran, Tehran University of Medical Science, Tehran, Iran; 3) Biotechnology Department, Pasteur Institute of Iran, Tehran, Iran.

Introduction: Cyclin D1 is a key regulator of cell cycle progression and a demonstrated oncogene. Over-expression of cyclin D1 is implicated in the pathogenesis of several cancers, including gastric cancer. Cyclin D1 regulates cell cycle progression by activating cyclin-dependent kinases 4 and 6 (CDK4 and CDK6), which in turn phosphorylates the retinoblastoma protein (pRb). This reaction inactivates pRb and is postulated to lead to progression through a G1S checkpoint, committing the cell to DNA replication. Single nucleotide polymorphisms, may modulate expression of cyclin D1 transcripts. Our aim in this study was to investigate the association of cyclin D1 (G870A) single nucleotide polymorphism with susceptibility to gastric cancer among a sample of Iranian patients and compares the results with normal group. **Methods:** We investigated the association between G870A polymorphism of the CDN1 gene and gastric cancer. 96 patients and corresponding control matched for their age, sex and ethnicity were enrolled. Genomic DNA was extracted and specific primers designed for the target region were used. RFLP was used to detect the presence or the absence of the polymorphism. **Results:** 36.5% of cases were of wild type genotype while 48% were heterozygous and 12.5% were of GG (polymorphic) genotype. The respective numbers for our controls were 30%, 45% and 25%. **Conclusion:** Our preliminary result shows that the G870A polymorphism of the cyclin D1 gene has no apparent association with gastric cancer among a sample of Iranian patients. Further investigation involving more patients and investigating other polymorphisms of this gene are required to delineate the true role of CDN1 polymorphisms in the pathogenesis of gastric cancer.

Cancer Genetic Counseling: the experience of three Brazilian Services. *E.I. Palmero¹, L. Kalakun², F.R. Vargas³, J.C.C. Rocha⁴, P. Ashton-Prolla^{1,2,5}.* 1) Post-Graduate Course in Genetics and Molecular Biology, UFRGS; 2) Service of Medical Genetics HCPA; 3) Oncogenetics Program, INCA; 4) Genetics Department, Hospital do Cancer A.C. Camargo; 5) Department of Genetics UFRGS.

Cancer Genetic Counseling (CGC) in Brazil is presently being provided by university centers and private clinics. This research aimed to study and compare the individual structure and characteristics of three different CGC services in the public arena, in different States of Brazil. The three services presented similarities, such as carrying out clinical and laboratory research; having specific professionals working together in the counseling process; evaluating individual and familiar risks for hereditary cancer; organizing the process of genetic counseling itself and offering genetic predisposition testing under the auspicious of research projects. All services presented differences in the type of population at risk being evaluated, reflecting mainly in the way people are being referred to these services. In one of them, the only non-symptomatic patients to have access to CGC are families of the patients being treated in the same hospital, whereas in the other two services of CGC, counseling is available to anyone who presents motivation and has a risk factor for hereditary cancer. In conclusion, public and private CGC services in Brazil are few and, generally, they are within tertiary hospitals which are primarily developed to treat already diagnosed cancer patients. Although in this framework there is a potential for cancer prevention of non-symptomatic families of a patient at risk for hereditary cancer, the CGC in these services follows a traditional retrospective model with several limitations. Passive screening of the whole population and highly complex tertiary institutions with restrictive costs are among them. This study emphasizes the importance of the way the oncology settings are structured in Brazil, as well as the need for collaborative studies covering the whole country, to obtain a comprehensive profile of the hereditary cancer syndromes in the Brazilian population. Support: HCPA, CAPES, CNPq.

HY1553, a novel CDK inhibitor isolated from *Streptomyces* sp., induces G1 arrest and apoptosis in HeLa cells. C. Lee, M. Kim, S. Moon, H. Lim, Y. Cho. Dept Medical Genetics, Hanyang Univ, Seoul, Korea.

Proliferative disorders, such as cancer, are recognized as diseases of the cell cycle. In the course of screening for a novel cell cycle inhibitor, HY1553 was found from the culture broth of *Streptomyces*. The molecular ion of HY1553 was identified at m/z 248(C₁₂H₁₆O₁₆).

Inhibition of cancer cell proliferation: The antiproliferative effects of HY1553 were assessed in four human cancer cells and normal lymphocytes using MTT assay. The growth of all cancer cells tested was inhibited in a concentration-dependent manner. The estimated IC₅₀ determined for HY1553 in these cancer cell lines ranged from 60 to 96.8 μ M. Interestingly, HY1553 demonstrated no antiproliferative effect on the normal lymphocyte cells used as control.

Inhibition of cell cycle regulation: The cell cycle analysis was performed on HeLa cells, after exposure to HY1553 at 32 μ M for 48 h. Flow cytometric analysis revealed an appreciable arrest of cells in G1 phase. The HeLa cell population increased gradually from 34% at 0 h, to 47% at 48 h in the G1 phase, after exposure to 32 μ M of HY1553. Accordingly, treatment with HY1553 induced a G1 phase arrest of the cell cycle progression of the HeLa cells by up-regulation of p21. Furthermore, HY1553 decreases the levels of CDK4/6, and cyclin D in Western blot analyses.

Induction of apoptosis: When HeLa cells were incubated with 80 μ M of HY1553 for 48 h, an apoptotic DNA fragmentation was observed in the HeLa cells using TUNEL assay. HY1553 induced up-regulation of Bax, and activation of procaspase-9. Furthermore, HY1553 induced proteolytic cleavage of procaspase-3 into its active form, and subsequent cleavage of PARP. The activation of caspase-9 is likely to occur via the mitochondria pathway which leads to cytochrome c release, followed by cleavage of procaspase-9.

The BOADICEA model of genetic susceptibility to breast and ovarian cancer: updating and validation. A.C.

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We previously derived a model of breast and ovarian cancer susceptibility using segregation analysis based on a population based series of 1484 breast cancer cases and 156 high-risk families from the UK. We have updated this model using additional data from two UK population based studies of breast cancer and family data from BRCA1/2 carriers identified in 22 population based studies of breast and ovarian cancer. The combined dataset includes 2785 families (301 BRCA1, 236 BRCA2 positive). According to the model, susceptibility to breast cancer is explained by mutations in BRCA1 and BRCA2 plus a polygenic effect (joint multiplicative effects of many genes of small effect). Incidence rates were smoothed using locally weighted regression techniques to avoid large variations between adjacent intervals. A birth cohort effect on the cancer risks is implemented, whereby each individual is assumed to develop cancer according to calendar period-specific incidence rates. The variance of the polygenic component declines with age, from 3.2 at age 30 to 1.3 at age 60. The predicted familial relative risks and BRCA1/2 prevalence among cases are close to those observed in population studies. The model predicts that the average breast cancer risks in BRCA1/2 carriers increase in more recent birth cohorts. For example, the average cumulative breast cancer risk to BRCA1 carriers is 50% for women born in 1920-29 and 58% for women born after 1950).

SISE matters: a method for measuring the information content of kinships when assessing the likelihood of HNPCC. *R.C. Green.* Memorial University, St. John's, NL, Canada.

Hereditary non polyposis colon cancer (HNPCC) is a significant cause of colorectal and other malignancies. The diagnosis of HNPCC relies on finding multiple cases of colorectal or other specific cancers within a family. In the absence of a significant family history, a diagnosis of HNPCC is seldom entertained. We postulate that small kinships with low information content are more likely to be designated at low risk of an inherited predisposition than are large kinships. This leads to under-diagnosis of HNPCC in small families and results in inadequate treatment, follow-up and family counselling. We have developed an objective quantitative measurement of the information content of individual pedigrees called the SISE coefficient (Sum of Information on Seventy year old Equivalents). The SISE coefficient of a family is a function of the number of informative relatives of the index case, of their ages and of the age-dependent penetrance of HNPCC mutations. For example a large family with a preponderance of young people and a SISE of 6.0 is as informative as a family where the index case has only 6 relatives, all first degree and older than 70 years. A population based series of 538 colorectal cancer cases was assessed by analysis of family history for the likelihood of carrying an HNPCC mutation and by measuring the SISE coefficient. Risk of HNPCC was designated as high (19 Amsterdam criteria families), intermediate (262 families) or low (257 families). Families with a low SISE coefficient were significantly more likely to be designated at low risk of HNPCC ($p < 0.001$). Using a cumulative binomial distribution function, we estimated the likelihood of observing multiple cancers in families of different SISE coefficients. In an HNPCC family with a SISE coefficient of 8.0 there is a 10% probability that < 2 HNPCC cancers will be observed (excluding the index case). In our series, 53% of "low risk" families had a SISE coefficient of 8.0 or less. The SISE coefficient can therefore be used to indicate the potential for a false negative diagnosis of HNPCC.

***BRCA1* and *BRCA2* mutations in an ethnically diverse cohort of high-risk women: evaluation of risk prediction models in African American and Caucasian families.** *H.J. Kim*¹, *R. Nanda*¹, *P.L. Schumm*², *S. Cummings*¹, *O.I. Olopade*¹. 1) Section of Hematology/Oncology, Department of Medicine, The University of Chicago, Chicago, IL; 2) Department of Health Studies, The University of Chicago, Chicago, IL.

To evaluate the performance of two risk prediction models, BRCAPRO and IBIS, in African American (AA) and Caucasian families (CA), we performed a retrospective analysis of genetic tests performed in our high-risk clinic from 1997 to date. Families included had at least 2 individuals with breast and/or ovarian cancers in 1st- and 2nd-degree relatives. For each family, the individual with the highest probability of being a mutation carrier based on the age of onset was selected for genetic testing. We used receiver operating characteristics (ROC) curve analyses to evaluate the performance of the models. There were 102 CA, 48 AA, 33 Ashkenazi Jewish (AJ), and 10 other minority families. This represents the largest cohort of ethnically diverse families reported to date. Excluding unclassified variants (UVs), genetic susceptibility to breast cancer could be explained by *BRCA1* mutations in 25.9% of all families, while 15.0% had *BRCA2* mutations. The mutation rate of *BRCA1* among AA was lower than in CA (14.6% vs 26.5%), while that of *BRCA2* was similar between the 2 groups (12.5% vs 13.7%). As expected, AA had the highest rate of UVs, while AJ had the highest mutation rates (45.5% for *BRCA1* and 24.2% for *BRCA2*). Among 6 Hispanic families one had a *BRCA2* mutation, and among 3 Asian families one had a *BRCA1* mutation. The likelihood of having a mutation in either gene was strongly associated with the number of breast/ovarian cancer cases in 1st- and 2nd-degree relatives and early age of onset of breast cancer. ROC curve analyses showed that BRCAPRO performed as well in AA as in other ethnic groups. Data from the IBIS model will be compared to BRCAPRO. Thus, *BRCA1/2* mutations occur in women of diverse ethnic origins, and genetic testing should be an integral part of comprehensive cancer risk management without regard to the ethnicity of the family. Future work will evaluate the clinical utility of different risk assessment tools in ethnically diverse populations seen in high-risk clinics in US, especially AA.

Patient Interest in Assessment of Familial Prostate Cancer Risk in Calgary, Alberta. *K. McLachlan¹, B. Donnelly^{2,3}, K. Carlson², J. Dushiniski², F. Elliott², W. Hyndman², G. Kozak², J. Lee², D. Metcalfe², P. Wilkin², R. Barr², R. Hughes³.* 1) Population Health Information, Tom Baker Cancer Centre, Calgary, AB, Canada; 2) Surgery, Univ. Calgary, AB; 3) Oncology, Univ. Calgary, AB.

This study examined the interest of men diagnosed with prostate cancer in undergoing a formal assessment of familial cancer risk. **SUBJECTS:** 1141 men from the Calgary region who had biopsies for prostate cancer diagnosis within the past 3 years. **METHODS:** Letters of invitation to participate in a prostate cancer followup study + familial cancer risk assessment were mailed together with a 1 page family history questionnaire. **RESULTS:** 431 men (37.8%) agreed to participate in the follow-up study including 283 men (24.8%) who provided familial cancer histories for genetic risk assessment. The family cancer histories were categorized into 3 groups: a) no risk (single case > 60 yr), b) possible risk (2 males with prostate cancer or one male diagnosed < 50 yr), c) probable (3 or more cancer diagnoses suggestive of hereditary prostate cancer HPC, or hereditary breast & ovarian cancer HBOC). The later 2 groups were asked complete a more detailed family history questionnaire for further assessment. **Results:** After review of the family history information, 28.8% were considered sporadic cases (82 men), 17.2 % (49 men) had a possible familial cancer risk and 54% (120 men) had family cancer histories suggestive of hereditary cancer syndromes (HPC, HBOC). The mean age of the men with possible or probable familial cancer risk was 58.8 yr + 10.5 yr SD. **CONCLUSION:** This population-based study found that approximately 11% of men diagnosed with prostate cancer in the Calgary region had family histories suggestive of hereditary cancer risk. Education (genetic counselling), risk reduction (vitamin E, selenium, cox-2 inhibitors), and early detection (DRE, PSA) may reduce the cancer-related morbidity and mortality for this group of high risk families. **Funding:** Alberta Health and Wellness, Alberta Cancer Board, Calgary Prostate Cancer Foundation.

Accuracy of Computerized BRCA1/2 Risk Assessment Models. *S.R. Nanda, N. Scanlan, G. Sheridan, P.J. Ainsworth, J.H. Jung, A. Sluyters.* Cancer Genetics, London Health Sciences Centre, London, ON. Canada.

Due to the increasing demand for BRCA1/2 genetic testing, breast cancer risk prediction models are being increasingly used. Cancergene version 3.4 (<http://www3.utsouthwestern.edu/cancergene/>) is a program that uses several mathematical risk assessment models (BRCAPRO, FHAT and Myriad II) to predict the probability of an individual carrying a BRCA1/2 mutation. The aim of this study was to determine the accuracy of these models in correctly predicting BRCA1/2 mutation status in our Southwestern Ontario population. The family histories of all probands seen through the Cancer Genetics clinic at London Health Sciences Centre, who underwent complete BRCA1/2 testing and had results available, were entered into Cancergene (n=667). A specific protocol was used to enter the pedigrees. From this, the probands personal risk of having a BRCA1/2 mutation was calculated using the models identified above. A family risk was determined by running the program on the highest risk individual in each family and based on Mendelian autosomal dominant inheritance the risk of the original proband was calculated using all three models. Among the probands (84.74% of whom were affected with cancer), 12.89% had a BRCA1/2 mutation and 87.11% did not test positive for a mutation. If a cut-off of 0.1 was used to determine testing eligibility, 37.21% of the positive probands and 65.82% of all probands would not have received testing based on their total BRCAPRO score. The sensitivity of the personal risk for total BRCAPRO was 62.79% and the specificity was 70.05%. The Myriad and FHAT models showed similar trends. Many limitations were encountered when using Cancergene. A specific limitation was that only first and second degree relatives could be entered into pedigrees. In addition, the program did not account for half siblings or consanguinity. This study suggests that one cannot solely rely on mathematical models for an accurate risk assessment. Clinical judgement is essential to establish a family's BRCA1/2 testing eligibility.

The genetic contribution of colorectal cancer in a Newfoundland region. *P.S. Parfrey¹, F. Curtis¹, S. Stuckless¹, A. Hyde², M.O. Woods², H.B. Younghusband², D. Robb³, R.C. Green², J.S. Green².* 1) Clinical Epidemiology, Memorial University of Newfoundland, St. John's, NL, Canada; 2) Genetics, Memorial University of Newfoundland; 3) Pathology, Memorial University of Newfoundland.

To determine the potential genetic contribution of colorectal cancer in the Avalon Peninsula, all patients < 70 years who presented with nonpolyposis colorectal cancer in 1997/8 (N=179) were identified and 79 provided detailed family histories. Nine probands (11%) had HNPCC, defined by Amsterdam I and II criteria; 51% of 79 family members had developed an HNPCC cancer by age 70 years. Thirteen (16%) families fulfilled Amsterdam II criteria modified for age (at least one cancer 60 years) and for cancer (HNPCC cancer defined by revised Bethesda); 30% of 116 members had developed cancer by age 70. 23 (29%) families were at intermediate risk based on revised Bethesda guidelines (excluding age/cancer modified group). Of 221 members, 10% had developed cancer by age 70. The low risk group was classified by informativeness of family structure, defined by the presence of at least 6 family members potentially at 50% risk of inheriting HNPCC who survived to 60 years or developed cancer. Nine (11%) families were uninformative; 8% of 61 members had developed cancer by age 70. The remaining 24 (30%) families were at low risk as confirmed by an informative family history. 3% of 294 members developed cancer by 70 years. The Hazard ratios for cancer in first and second degree relatives of probands compared to the low risk informative group, were 21.1(95% CI-10.0-44.6) for Amsterdam I + II, 10.9(CI 5.3-22.3) for age/cancer modified families, 3.8(1.8-8.3) for revised Bethesda criteria, and 1.8(0.5-6.6) for low risk uninformative families. 29% of probands from families defined by Amsterdam I and II demonstrated microsatellite instability, as did 33% of those from age/cancer modified group, 23% of revised Bethesda, and 14% of low risk group. In this population based study it is likely the genetic contribution of nonpolyposis colorectal cancer in Newfoundland is high, and that mutations in novel genes, associated with microsatellite stability, predispose to HNPCC.

Classification of unclassified variants in *BRCA1* and *BRCA2*. A. Spurdle¹, S. Healey¹, A. Bekessy^{1,5}, A. Marsh¹, kConFab², S. Tavtigian³, D. Goldgar³, P. Waring², M. Jenkins⁴, A. Tesoriero⁴, M. Southey⁴, J. Hopper⁴, P. Lovelock^{1,5}, M. Brown⁵, G. Chenevix-Trench¹. 1) Cancer & Cell Biology Division, Queensland Inst Med Research, Brisbane, QLD, Australia; 2) Peter MacCallum Cancer Institute, Melbourne; 3) International Agency for Research into Cancer, Lyon; 4) Centre for Genetic Epidemiology, University of Melbourne; 5) Department of Biochemistry, University of Queensland, Brisbane.

Many *BRCA1* and *BRCA2* sequence variants found in multiple-case breast cancer families are missense variants of unknown pathogenicity, termed unclassified variants (UVs). These create a dilemma for genetic counseling, and constitute about 8% of *BRCA1* and 18% of *BRCA2* variants detected among families ascertained by kConFab, an Australian national consortium for familial breast cancer research. We have started a genetic and histopathological analysis of these UVs, aiming to classify some variants as neutral/low clinical significance and to prioritize the rest for functional assays to assist in classification. Our approach has been to: a) evaluate the variant frequency in controls; b) undertake *in silico* analyses to investigate evolutionary conservation of sequence, and possible impact on structure or splicing; c) genotype as many family members as possible for penetrance and Bayesian causality analyses; d) examine tumours for loss of variant or wild type allele; e) use histopathological analysis to identify tumours with a *BRCA1*-like phenotype. Results from preliminary analysis of 10 *BRCA1* and 16 *BRCA2* UVs indicate that at least 5 *BRCA1* and 12 *BRCA2* variants are unlikely to act as high-risk deleterious alleles since they occur commonly in control individuals without cancer, and/or are absent in multiple affected family members, and/or show loss of the variant in tumour tissue. In the subset of 5 *BRCA1* variants considered unlikely pathogenic, 4/4 variants examined did not exhibit a *BRCA1*-like histopathological phenotype, and cross-species sequence comparison supported neutrality for the 5th variant. These data suggest that a combination of genetic, histopathological and evolutionary approaches will facilitate assessment of the pathogenicity of *BRCA1* and *BRCA2* UVs.

Homozygosity for *RNASEL* R462Q increases the risk of sporadic prostate cancer in Ashkenazi Jewish men. L.

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Ribonuclease L (*RNASEL*) is involved in interferon antiviral activities and cell proliferation and presumably functions as a tumor suppressor gene. Alterations in the *RNASEL* gene have been implicated as predisposing factors in hereditary prostate cancer (HPC), while the genetic influences on sporadic prostate cancer (SPC) remain largely unknown. We genotyped 228 SPC patients of Ashkenazi Jewish (AJ) descent and 1,008 unrelated AJ healthy controls for known alterations in *RNASEL* including the R462Q variant that has 30% of wild-type enzyme activity, the AJ frameshift mutation 471delAAAG, and the E265X nonsense mutation, all of which have been reported to be associated with HPC. Importantly, after subtracting the 471delAAAG carriers, the frequency of R462Q homozygosity in AJ males with SPC was more than twice that of the controls and was statistically significant (13.6%; vs. 6.2%; OR = 2.40; P = 0.004), suggesting that R462Q homozygosity is a predisposing factor for the development of SPC. Neither 471delAAAG (q=0.024) nor the E265X (q=0.002) were found to have increased incidence in the AJ SPC patients. Genotyping of polymorphic markers tightly linked to the *RNASEL* locus on 1q25 indicated that R462Q was present on multiple distinct haplotypes in the AJ controls and SPC patients. These findings suggest that R462Q did not originate from a single founder in the AJ population and are consistent with the increased cancer predisposition associated with R462Q homozygosity. The high frequency of R462Q homozygosity in the AJ population (8%) as well as other populations, including Caucasian (~11%) and African American (~1%), suggest that this substitution may be a common risk factor for prostate cancer and that distinguishing between HPC and SPC with such alleles may be difficult, as it would depend on the genotype of close male relatives and the penetrance of the phenotype.

X chromosome inactivation in blood and tumor from females with colorectal cancer. *G.P.S. Knudsen¹, J. Pedersen¹, L. Thorstensen², T. Lvig³, R.A. Lothe^{2,4}, G.I. Meling⁵, K.H. rstavik^{1,6}*. 1) Department of Medical Genetics, Faculty division RH, University of Oslo, Norway; 2) Department of Genetics, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway; 3) Institute of Forensic Medicine, University of Oslo, Rikshospitalet, Norway; 4) Department of Molecular Biosciences, University of Oslo, Norway; 5) Department of Surgery, Akershus University Hospital, University of Oslo, Nordbyhagen, Norway; 6) Department of Medical Genetics, Rikshospitalet, Oslo, Norway.

X chromosome inactivation occurs in early embryonic life and leads to a random X inactivation pattern in most females. Skewed X inactivation may arise due to chance, genetic factors or as a result of selection in favour or against cells with a specific genotype. X inactivation analysis has been used for clonality assessment of various tumors. A monoclonal tumor consists of cells with the same X chromosome as the active X, and thus shows a skewed X inactivation pattern. Colorectal carcinomas have been reported to be of both monoclonal and polyclonal origin. We investigated the clonality of colorectal carcinomas in females and compared X inactivation patterns in tumors with that of blood to see if monoclonality might be biased by constitutional skewed X inactivation. Informative X inactivation results were obtained by assessment of the methylation status of the androgen receptor in blood and tumor from 74 female colorectal cancer patients. We found no difference in frequency of skewed X inactivation (defined as skewed when 80 % of the cells have a preferential inactivation of one of the two X chromosomes) in blood between patients (39%) and controls (36%), but the frequency of skewed X inactivation was higher in blood than in tumor (16%) ($p < 0.001$). This indicates a polyclonal origin of the tumors and no biased monoclonality. We found skewing more frequently in tumors with microsatellite stability (11 of 59) than in tumors with microsatellite instability (none of 14) ($p = 0.109$). This might indicate that the hypermethylation of various promoters associated with microsatellite instability also affects the androgen receptor locus on the X chromosome.

Penetrance estimates of deleterious BRCA1 and BRCA2 mutations in high-risk French Canadian families. F.

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We used data from 251 French Canadian families with multiple affected individuals to estimate age-specific breast and ovarian cancer risks in BRCA1 and BRCA2 mutation carriers. Families were included in the analyses if an initial mutation carrier was identified in the family, and at least one other family member had been tested subsequently. There were 25 eligible families with BRCA1 mutations and 28 families with BRCA2 mutations. We estimated log-relative risks of breast and ovarian cancer in mutation carriers compared to the population risks by maximum likelihood, using a modified segregation analysis implemented in the computer program MENDEL. We maximised the conditional likelihood of all phenotypic and genotypic information in the family given all disease phenotypes and the genotypic information up to the point where the first mutation carrier was identified. The cumulative risk of breast cancer by age 70 in BRCA1 mutation carriers was estimated to be 91% (95% CI: 36-99%) and the ovarian cancer risk 9% (0-41%). The corresponding risks in BRCA2 mutation carriers were 84% (0-98%) and 37% (0-78%). The estimated breast cancer risks are in line with those found in multiple-case families, but somewhat higher than those obtained from the meta-analysis of families of BRCA1/2 carriers identified through population-based studies (Antoniou et al (2003), AJHG 72:1117). The BRCA1 ovarian cancer risk is also lower than previously reported. However, none of these estimates is significantly different from the meta-analysis estimates and thus our data are in agreement with those previously reported in other populations.

Genome-Wide Discovery of Loci Influencing Chemotherapy Cytotoxicity. *J. Watters*¹, *A. Kraja*², *M. Meucci*¹, *M. Province*², *H. McLeod*¹. 1) Dept of Medicine, Washington Univ, St Louis, MO; 2) Dept of Biostatistics, Washington Univ, St Louis, MO.

While numerous genes are likely to influence chemotherapy response, current candidate gene-based pharmacogenetics approaches require a priori knowledge and the selection of a small number of candidate genes for hypothesis testing. In this study, a novel *ex vivo* familial genetics strategy utilizing lymphoblastoid cells derived from the CEPH reference pedigrees was employed to discover genetic determinants of cytotoxicity to the mechanistically distinct chemotherapy agents 5-fluorouracil and docetaxel. The sensitivity of CEPH cells to drug-induced cell death was shown to be similar to that observed in the NCI-60 panel of tumor cell lines, and 5-fluorouracil and docetaxel were shown to cause apoptotic cell death involving caspase-3 cleavage in CEPH cells. Cytotoxicity to 5-fluorouracil and docetaxel were shown to be heritable traits in CEPH families, with heritability values ranging from 0.26 to 0.65 for 5-fluorouracil and 0.21 to 0.70 for docetaxel, varying with dose. Genome-wide linkage analysis was also used to map a QTL influencing the cellular effects of 5-fluorouracil to chromosome 9q13-q22 (LOD=3.44), and two QTLs influencing the cellular effects of docetaxel to chromosomes 5q11-21 (LOD=2.21) and 9q13-q22 (LOD=2.73). Utilizing SNP genotype data provided by the SNP consortium and the International HapMap project, family-based tests for association were employed to identify positional candidate genes. Finally, whole-genome expression profiling was used to identify candidate genes that were differentially expressed between resistant and sensitive cell lines. Alteration of gene expression by siRNA and confirmation in an independent sample set are currently being used to validate candidate genes. This study identifies genomic regions and candidate genes likely influence chemotherapy cytotoxicity using a combination of genome-wide linkage analysis, SNP association tests, and gene expression profiling, thus providing a widely applicable strategy for pharmacogenomic discovery without the requirement for a priori candidate gene selection.

A *Prkar1a* down-regulation in vitro system for the study of protein kinase A-related tumorigenicity in mouse cells and in Carney complex. *F.D. Weinberg, K.J. Griffin, A. Binstock, A.J. Bauer, L. Matyakhina, C.A. Stratakis.* National Institutes of Health, Bethesda, MD.

Carney Complex (CNC) is a multiple neoplasia syndrome associated with skin pigmentation defects and other abnormalities. The *PRKARIA* gene, encoding the type 1 regulatory subunit of protein kinase A (PKA), is mutated in approximately half of the cases and may function as a tumor-suppressor gene. To establish an animal model specific to CNC, heterozygote *Prkar1a* +/- mice were generated using flanking loxP sites. Mouse embryonic fibroblast (MEF) cell lines were generated at 13 days post-conception. Down-regulation of the *Prkar1a* gene was attempted in these cells using short interfering RNAs (siRNA) specific to the gene. First, a control siRNA against vinculin led to down-regulation of the vinculin protein in these MEFs. After transfection with the siRNA directed against *Prkar1a*, down-regulation of *Prkar1a* at the mRNA level was also shown; interestingly, this affected the mRNA levels of the other subunits (*Prkar1b*, *Prkar2b*, *Prkar2a*, and *Prkaca*). Protein levels of *prkar1* were also decreased along with corresponding changes in the protein levels of the other PKA subunits. *Prkar1a* -/- MEFs are spontaneously immortalized; current work is aiming at elucidating growth and proliferation characteristics of *Prkar1a* +/- cells at baseline and after further down regulation of the *Prkar1a* gene; cell cycle, proliferation and morphology experiments are currently pending in cells transfected with the siRNAs. We conclude that we have generated a system of *Prkar1a* down-regulation employing siRNAs in proliferating, primary MEFs. Future studies will use these cells in an attempt to link *Prkar1a* down-regulation with the abnormal growth and proliferation phenotype of human and mouse mesenchymal and other cells.

Renal oncocytomas as a model to study the regulation of mitochondrial proliferation. *S. Zanssen¹, X. Hong¹, E. Kessel¹, B. Gunawan², L. Fuzesi², D. Warburton^{3,4}, E. Schon^{1,4}.* 1) Department of Neurology, Columbia University, 630 West 168th Street, New York, NY 10032, USA;; 2) Department of Pathology, University of Goettingen, Germany; 3) Department of Pediatrics, Columbia University, 630 West 168th Street, New York, NY 10032, USA;; 4) Department of Genetics and Development, Columbia University, 630 West 168th Street, New York, NY 10032, USA;.

Mitochondrial defects have been associated with severe neurodegenerative disorders with human cancers. A variety of encephalomyopathies have been linked to alterations of mtDNA. The predominantly neurological symptoms are not produced by the mutations per se, but by an unregulated proliferation of pathologic mitochondria. We are using oncocytomas, which are mostly benign epithelial tumors whose predominant feature is a massive accumulation of mitochondria in the cytoplasm, as a way to search for unknown genes that play a role in regulation of mitochondrial proliferation. We studied three oncocytomas with 11q13 rearrangements. Fluorescent in situ hybridization (FISH) using BAC clones in the region showed that the 11q13 breakpoints in all three tumors are near the CCND1 (BCL1) gene. None of the breakpoints disrupts any known gene other than CCND1, but gene prediction programs suggest several human genes in the breakpoint region. The rearrangement in one tumor consisted of a segmental duplication that included 11q13, presenting several mitochondrially-targeted genes immediately distal to CCND1 as interesting candidate genes.

MICROTIA AND AURAL ATRESIA. AUDIOLOGICAL FINDING. *F. Castillo-Lorca*¹, *M. Diaz-Garcia*², *G. Garcia-Sanchez*¹, *A. Garcia-Huerta*¹, *L. Hernandez-Gomez*³, *C.F. Martinez-Cruz*^{4,5}. 1) Departamento de Genetica. Instituto de la Comunicacion Humana. Centro Nacional de Rehabilitacion SSa. Mexico; 2) Departamento de Investigacion. Instituto de la Comunicacion Humana. Centro Nacional de Rehabilitacion SSa. Mexico; 3) Servicio de Audiologia. Instituto de la Comunicacion Humana. Centro Nacional de Rehabilitacion SSa. Mexico; 4) Servicio de Comunicacion Humana. Departamento de Seguimiento Pediatrico. Instituto Nacional de Perinatologia SSa. Mexico; 5) Servicio de Pediatria. HGZ No.53 Los Reyes la Paz. Instituto Mexicano del Seguro Social. Mexico.

Microtia is a birth defect that affects one in 8000-10,000 children. It typically affects males and is more frequent on the right ear. The microtia happens in the first trimester of pregnancy while the ears are developing. In some cases it is hereditary. Aural atresia is the absence of an opening for the ear canal. It doesn't mean that there is no canal, but rather that it is not open to the outside. The rate of occurrence is 1 in 10,000 to 1 in 20,000 live births. Unilateral atresia is three times more common than bilateral atresia, more common on the right side, and males are more commonly affected. Associated with a variety of syndromes and disorders as well as occurring in isolation. Many investigations point that microtia with atresia cause severe conductive hearing loss. We studied 130 patients with different microtia types, with or without atresia, 79 men (60.8%) and 51 women (39.4%). With mean age of 9.36 years. All patients were tested with conventional audiometry. The most frequent findings were: Microtia-Atresia type II (68.8 % right and 65.8 % left side); with regard to hearing loss it was of conductive type (86.9%), severe (92.8 %), ascending (67.5 %). All the ears with microtia-atresia showed hearing loss and only 72 % with stenosis. We didn't find association between gender and severity of the microtia; severity and side of the microtia, malformation of the auditory external canal and affected side, X² (P> 0.05). In the present study 99.2 % of the patients with microtia and aural atresia had some degree of hearing loss in the affected ear.

BRANQUIO-OTO-RENAL SYNDROME IN MEXICAN PATIENTS. A. Garcia-Huerta¹, A. Alcala Vazquez¹, C.F. Martinez-Cruz^{2,3}, L. Hernandez-Gomez⁴, F. Castillo-Lorca¹, G. Garcia-Sanchez¹. 1) Departamento de Genetica. Instituto de la Comunicacion Humana. Centro Nacional de Rehabilitacion. Mexico; 2) Servicio de Comunicacion Humana. Departamenmto de Seguimiento Pediatrico. Instituto de Perinatologia. Mexico; 3) Servicio de Pediatria. HGZ No.53 Los Reyes la Paz. Instituto Mexicano del Seguro Social. Mexico; 4) Servicio de Audiologia. Instituto de la Comunicacion Humana. Centro Nacional de Rehabilitacion. Mexico.

Branchio-oto-renal syndrome is the second most common type of autosomal dominant syndromic hearing loss. It consists of conductive, sensorineural, or mixed hearing loss in association with branchial cleft cysts or fistulae, malformations of the external ear including preauricular pits, and renal anomalies. Penetrance is high, but expressivity is extremely variable. Approximately 40% of persons with the BOR phenotype have mutations in the EYA1 gene (chromosomal locus 8q13), suggesting that BOR may be caused by mutations at other loci. Molecular genetic testing is available clinically. We analyzed 8 patients with branchio-oto-renal syndrome. 6 had branchial cysts or fistulas; 4 were bilateral; 2 had preauricular pits, 1 with auricular appendage and 2 cases of low set ears. We had 3 cases of bilateral microtia; 1 bilateral malformation of the ossicles and 1 bilateral malformed cochlea. We found hemifacial microsomia, facial palsy, mandibular hypoplasia and submucous cleft palate, among others. They did not have renal malformations. All patients had hearing loss, mainly sensorineural. The family history was negative in all patients. Therefore, it would be interesting to carry out genetic molecular testings, cause here in Mexico does not exist similar studies in patients with BOR.

VAN DER WOUDE SYNDROME. IN A MEXICAN FAMILY. *G. Garcia-Sanchez¹, C.F. Martinez-Cruz^{2,3}, A. Garcia-Huerta¹, F. Castillo-Lorca¹, L. Hernandez-Gomez⁴, M.R. Baez-Reyes⁵*. 1) Departamento de Genetica. Instituto de la Comunicacion Humana. Centro Nacional de Rehabilitacion. Mexico; 2) Servicio de Comunicacion Humana. Departamenmto de Seguimiento Pediatrico. Instituto Perinatologia. Mexico; 3) Servicio de Pediatria. HGZ No.53 Los Reyes la Paz. Instituto Mexicano del Seguro Social. Mexico; 4) Servicio de Audiologia. Instituto de la Comunicacion Humana. Centro Nacional de Rehabilitacion SSa. Mexico; 5) Servicio de Genetica. Departamento de Investigacion. Instituto Nacional de Perinatologia SSa. Mexico.

Van der Woude syndrome (VWS) is a dominantly inherited developmental disorder characterized by pits and/or sinuses of the lower lip, cleft lip/palate (CL/P), cleft palate (CP), bifid uvula, and hypodontia. The expression of VWS, which has incomplete penetrance, is highly variable. It is the most common cleft syndrome. Most reported cases of VWS have been linked to chromosome 1q32-q41 (VWS1), but a second VWS locus (VWS2) has been mapped to 1p34, performed direct sequence analysis of genes and presumptive transcripts in the 350-kb VWS critical region identified by linkage analysis and identified mutations in the gene encoding interferon regulatory factor-6 (IRF6). Mutation analysis demonstrated that popliteal pterygium syndrome can be caused by mutations in the same gene and is therefore allelic to van der Woude syndrome. We report a Mexican family with 6 affected members with VWS in 3 generations (autosomal dominant inheritance). We found the proband, his grandfather and paternal uncle with lip pits, his father had unilateral cleft lip and palate. His sister had cleft palate and his brother had bifid uvula. All three clinical features occurred together in an affected individual. This family has all the VWS features, proving the variable expressivity of the syndrome. Molecular testings are outstanding.

RADIOLOGICAL FINDINGS IN SPINE OF PATIENTS WITH MICROTIA AND AURAL ATRESIA. L.

Hernandez-Gomez¹, S.G. Juarez-Garcia², G. Garcia-Sanchez², M. Diaz-Garcia³, C.F. Martinez-Cruz^{4,5}. 1) Servicio de Audiología. Instituto de la Comunicación Humana. Centro Nacional de Rehabilitación SSA. Mexico; 2) Departamento de Genética. Instituto de la Comunicación Humana. Centro Nacional de Rehabilitación SSA. Mexico; 3) Departamento de Investigación. Instituto de la Comunicación Humana. Centro Nacional de Rehabilitación SSA. Mexico; 4) Servicio de Comunicación Humana. Departamento de Seguimiento Pediátrico. Instituto Nacional de Perinatología SSA. Mexico; 5) Servicio de Pediatría. HGZ No.53 Los Reyes la Paz. Instituto Mexicano del Seguro Social. Mexico.

Microtia is a birth defect that affects one in 8000-10,000 children. It typically affects males and is more frequent on the right ear. The microtia happens in the first trimester of pregnancy while the ears are developing. In some cases it is hereditary. Aural atresia is the absence of an opening for the ear canal. It doesn't mean that there is no canal, but rather that it is not open to the outside. The rate of occurrence is 1 in 10,000 to 1 in 20,000 live births. Unilateral atresia is three times more common than bilateral atresia, more common on the right side, and males are more commonly affected. Associated with a variety of syndromes and disorders as well as occurring in isolation. We studied 57 Mexican patients with different microtia types, 60% men (n=34) and 40% women (n=23) with an age average of 2 to 17 years. 31.5% (n=18) with right microtia, 17.5% (n=10) with left microtia and 51% with bilateral microtia. Microtia type I in 39% (n=22) and types II-III in 61% (n=35). With regard to the presence of spine malformations in the x-rays, 33% (n=19) had any malformation, and 67% had not. Statistically significant (Fisher exact test $P < 0.10$).

STICKLER SYNDROME. A MEXICAN FAMILY REPORT. *C.F. Martinez-Cruz^{1,2}, G. Garcia-Sanchez³, A. Garcia-Huerta³, F. Castillo-Lorca³, L. Hernandez-Gomez⁴.* 1) Servicio de Comunicacion Humana. Departamento de Seguimiento Pediatrico. Instituto Nacional de Perinatologia SSa. Mexico; 2) Servicio de Pediatria. HGZ No.53 Los Reyes la Paz. Instituto Mexicano del Seguro Social. Mexico; 3) Departamento de Genetica. Instituto de la Comunicacion Humana. Centro Nacional de Rehabilitacion SSa. Mexico; 4) Servicio de Audiologia. Instituto de la Comunicacion Humana. Centro Nacional de Rehabilitacion SSa. Mexico.

Stickler syndrome consists of progressive sensorineural hearing loss, cleft palate, and spondyloepiphyseal dysplasia resulting in osteoarthritis. Three types are recognized, based on the molecular genetic defect: STL1 (COL2A1), STL2 (COL11A2) and STL3 (COL11A1). STL1 and STL3 have severe myopia, which predisposes to retinal detachment, but this aspect of the phenotype is absent in STL2 because the COL11A2 gene is not expressed in the eye. Causative mutations have been found in the genes causing STL1, STL2, and STL3. Molecular genetic testing is available clinically. We report a Mexican family in which father and his two children are affected (autosomal dominant inheritance). All three patients had sensorineural bilateral profound, prelingual hearing loss, the younger child had severe myopia, the father and his elder child had retinal detachment and the younger child had cleft palate. In this family the Stickler syndrome is probably due to mutations in anyone of the genes STL1 or STL3.

Recognition of patterns of congenital syndromes through analyzing data from the OMIM database. X. Ji¹, J. Li-Ling². 1) Laboratory for Conservation and Utilization of Bio-resources, Yunnan University, Kunming 650091, CHINA; 2) Department of Medical Genetics, China Medical University, Shenyang 110001, CHINA.

Clustered features seen in various congenital syndromes may provide important information about mechanisms of embryonic development and pathogenesis of many isolated features. Summary of the patterns of such syndromes may also provide unique clues for the construction of genetic networks. In this study, clinical synopses of more than 4,500 syndromes (containing more than 50,000 individual features), as summarized by the Online Mendelian Inheritance in Man (OMIM) database (version of 15th March 2004), were extracted, checked and classified manually based on medical knowledge. Subsequently, statistical analysis was performed to identify the correlations between OMIM entries, clinical synopses, and their underlying genetic defects. An effort was also made to correlate the patterns of such syndromes with mechanisms of embryonic development. According to which feature or up-level category it was classified into, each OMIM entry was assigned a feature- or category-classification profile. Such profiles were then compared with each other to produce a similarity/distance matrices. Finally, hierarchical trees were constructed with neighbor-joining method based on the distance matrices (Detailed results may be viewed at: <http://www.bioinfo.tsinghua.edu.cn/~rich/science/omim/01/index.html>). Utilization of bioinformatics approaches such as pattern recognition and phylogenic trees has recognized particular patterns of disease occurrence. These have included midline defects, midline-radial ray abnormality, neurology-endocrine-immunity association, etc. Our analysis seems mostly fit the generalization made by Opitz, that most features seen in congenital syndromes are of midline defects, and that genetic defects in various forms and scales may cause congenital syndromes in a similar pattern. Notably, data from OMIM are probably only partial due to insufficient data, observational bias and natural selection. Our analysis of the OMIM, 'Phenotypic companion to the HGP', may provide a foundation for further studies into this direction.

Omodysplasia: report of the first Brazilian case. *L.M.J. Albano¹, L.A.N. Oliveira², D.R. Bertola¹, A.C. Paula¹, T.P. Delboni¹, C.A. Kim¹.* 1) Genetics Clinic Unit, Inst da Crianca, Hosp das Clinicas, Sao Paulo, Brazil; 2) Radiology, Instituto da Crianca, HC-FMUSP, Sao Paulo, Brazil.

Omodysplasia, an heterogeneous disorder with an autosomal recessive and dominant pattern of inheritance, constitutes an osteochondrodysplasia characterized by craniofacial dysmorphisms (depressed nasal bridge, broad base of the nose, long philtrum), shortened humeri and femora and a characteristic radial dislocation with radioulnar diastasis. The autosomal dominant form seems to be much rarer than the recessive one with abnormalities limited to the upper limbs, and the recessive presents a generalized involvement of the limbs and severe dwarfism. Both types share the facial characteristics, the typical defect of the distal humerus and the complex deformity of the elbows. Less than 25 recessive cases were reported in the literature so far. We report the first male Brazilian recessive case complaining of pain in his shoulders, hip and legs: a 9y3mo-old boy from healthy and consanguineous parents presenting short stature, frontal bossing, depressed nasal bridge, small nose and midfacial flatening, shortened arms, limited extension of the elbows and knees, tiptoe gait, intellectual impairment and motor development delay. Skeletal survey showed shortened humeri bilaterally with abnormal sloping of the distal articular surface, shallow olecranon fossae with partially subluxed radii and radioulnar diastasis; short femora with metaphyseal widening and small laterally displaced patellae. Our case reinforces the findings stressed by some authors that the recessive form is the most common type, there is a male preponderance, and metacarpal shortening was not observed in recessive cases. As the facial features of omodysplasia overlap with achondroplasia and Robinow syndrome these conditions should be considered in the differential diagnosis as well as other ryzomelic dwarfisms.

Tibial camptomelia, oligodactyly and fibular aplasia in a male newborn. *W. Courtens¹, A. Jespers², I. Harrewijn², D. Puylaert³, F. Vanhoenacker⁴.* 1) Medical Genetics, University Hospital Antwerp, Antwerp,Belgium; 2) Department of Neonatology, University Childrens Hospital Queen Paola, Antwerp,Belgium; 3) Department of Orthopedics, University Childrens Hospital Queen Paola, Antwerp,Belgium; 4) Department of Radiology, University Hospital Antwerp, Antwerp, Belgium.

We report on a male newborn with a rarely described congenital limb deficiency syndrome consisting of an anterior bowing of the right lower limb at the distal third of the tibia with associated overlying soft tissue dimpling, oligodactyly of the right foot (presence of only 4 toes; the Vth ray was lacking), and a left-sided oligosyndactyly of the hand: there was a nearly complete syndactyly of fingers I-II and IV-V, the presence of only 4 metacarpal bones, the presence of only two distal phalanges of the IVth finger (attached to the Vth finger) ; and a cutaneous syndactyly between fingers III and IV-V, extending until the proximal interphalangeal joint. The right hand and left lower limb were clinically normal. At X-ray examination absence of the right fibula, absence of the right-sided Vth metatarsal and phalanges, and bowing and shortening of the right-sided tibia were noted. The femorae, humerae, ulnae, and radii were normal. The infant had no facial dysmorphism (except a mild micrognathia), nor other associated anomalies. A limb deficiency syndrome comparable to this case, has been reported by Hecht and Scott (1981) in half-sibs and was recognized as limb deficiency-heart malformation syndrome in OMIM [246570]. No other cases with a diagnosis of a similar syndrome were reported in this database so far. We found another two reports describing three cases comparable to our case and reviewed these cases. Since there was no associated heartdefect, except in one case, and since they all had three major findings in common, we propose to name it fibular aplasia-oligosyndactyly-tibial camptomelia syndrome. Additional case reports are needed to further delineate this rare limb deficiency syndrome.

Ollier disease. Report of the first Mexican familial case. *R. E' Vega¹, I.P. Davalos^{2,3}, L.E. Figuera^{2,3}, N.O. Davalos^{3,4}.* 1) Servicio de Traumatología y Ortopedia, Hospital Regional Valentin Gomez Farias, ISSSTE; 2) Division de Genetica, CIBO, IMSS; 3) Doctorado en Genetica Humana, CUCS, Universidad de Guadalajara; 4) Servicio de Genetica, Hospital General Regional Valentin Gomez Farias, ISSSTE. Guadalajara, Jalisco, Mexico.

The Ollier Disease (OD) or Multiple Enchondromatosis [OMIM #166000] is a rare skeletal disease, it was first described by Ollier as a chondrodysplasia. Enchondromas, characteristics of OD, are common benign cartilage tumors, that can occur as solitary lesions or as multiple lesions, they can occur anywhere, but are most common in the hands. OD can be present at birth, but it may not become apparent until early childhood. Enchondromas involving long bone are common, leading to progressive skeletal deformities and pathologic fractures with . It occurs in all races with no sex predominance. The condition is sporadic although 5 familial occurrences of Ollier disease have been reported. CASE REPORT: The propositus 74 male years-old. Clinically showed asymmetry in length and shape of involved limb. Radiologically revealed irregular, elongated tumorous radio lucent defects in metaphyses of tubular bones. Short tubular bones in hands and feet. The purpose of this paper is to describe a first familial case with OD with 17 affected members in 6 generations (M:11; F:6) suggesting an autosomal dominant inheritance with variable expressivity. All affected revealed skeletal anomalies such as pathologic fracture, irregular calcification within enchondromas lesions. Enchondromas are usually in or surrounding the growth-plate cartilage. It has been suggested that the defect can result from abnormal regulation of proliferation and terminal differentiation of chondrocytes in the adjoining growth plate. OD could be caused by a mutation in the PTH/PTHrP type I receptor (OMIM #166000). Gene map locus 3p22-p21.1. It remains to do molecular analysis on this family in order to determine if its defect is linked to this locus.

FIRST FAMILIAL CASE OF CANTU CRANIOFACIOCARDIOSKELETAL SYNDROME. *C.L. Garcia-Gonzalez^{1,3}, D. Garcia-Cruz^{2,3}, M.G. Lopez-Cardona^{1,3}, J.M. Cantu³, G. Castañeda-Cisneros^{1,3}, L.E. Figuera^{2,3}, J. Sanchez-Corona^{1,3}.* 1) Medicina Molecular, CIBO, IMSS, Guadalajara, Jalisco, Mexico; 2) Genetica, CIBO, IMSS, Guadalajara, Jalisco, Mexico; 3) Doctorado Genetica Humana, CUCS, U de G; Guadalajara, Jalisco, Mexico.

In 1982, some of us (Clin. Genet. 22: 172, 1982) described a craniofaciocardioskeletal dysmorphic syndrome in four unrelated girls, which was later catalogued as Cantu syndrome (MIM N 114620). It is characterized by mild mental retardation, short stature, macrocranium, prominent forehead, hypertelorism, exophthalmos, cardiac anomalies, cutis laxa, wrinkled palms and soles, joint hyperextensibility, wide ribs, and small vertebral bodies. The cases were all sporadic. The parents were nonconsanguineous. The father's age at the birth of each case was above 45, suggesting a dominant de novo mutation. We recently studied a girl aged 6 10/12 years who showed a similar syndrome in which the mother, maternal grandfather and brother presented similar facial and/or clinical characteristics. At the proposita's birth was detected hypoxia and suspected cardiopathy. Her psychomotor development has been delayed. Clinically she presented macrodolichocephaly, scarce hair with some alopecic areas, peculiar facies with prominent forehead, scarce eyebrows, antimongoloid palpebral fissures, short neck, low-set posterior hair, barrel-shaped thorax, nipple hypertelorism, wrinkled palms and soles, clitoris hypertrophy, hypoplastic labia majora, hyperelastic skin, joint hyperextensibility, wide ribs and small vertebral bodies. The doppler colour echocardiogram revealed a mild pulmonary stenosis. A cranial CAT showed megalencephaly, arachnoidal cysts and a skin biopsy increased elastic fibres. This is the first familial report of the Cantu craniofaciocardioskeletal syndrome. Some imagenological and histological features not previously described in this syndrome are also reported.

New radiological features in Kabuki (Niikawa-Kuroki) syndrome. *D. Genevieve¹, D. Sanlaville¹, M. Le Merrer¹, J. Amiel¹, P. Parent², S. Lyonnet¹, A. Munnich¹, V. Cormier-Daire¹.* 1) Departement de Genetique, Hopital Necker-Enfants Malades, Paris, France; 2) Service de Genetique, Hopital de Brest, Brest, France.

Kabuki syndrome (KS) is a rare multiple congenital anomaly/mental retardation syndrome with an estimated frequency of 1/32,000 in Japan. Five major criteria delineate KS namely characteristic facial dysmorphism, postnatal short stature, moderate mental retardation, dermatoglyphic and skeletal anomalies. Classical skeletal features are observed in largely 98% of cases and include scoliosis, sagittal cleft of vertebral body, short fifth finger with short middle phalanx, short metacarpal, coarse carpal bone, cone shaped epiphysis and advanced or delayed bone age. Here, we report on a series of 12 sporadic KS patients and focus on previously unreported radiological features namely abnormal shaped, round and irregular middle phalanx of the fifth finger (6/12), delayed bone age (8/12), flat superior and inferior femoral epiphyses (6/9), irregular internal femoral inferior epiphysis (5/9) and long and thin femoral neck (9/10). These radiological features, in combination with the previously skeletal anomalies reported in KS, can be used as helpful diagnostic criteria in KS.

COL2A1 mutation in spondyloepiphyseal dysplasia with hydrocephalus and pulmonary hypertension. *J. Hoover-Fong*¹, *I. Berkowitz*², *J. Hyland*³, *R. Lachman*⁴. 1) Inst Genetic Medicine, Greenberg Ctr Skeletal Dysplasias, Johns Hopkins U, Baltimore; 2) Johns Hopkins U, Baltimore; 3) Tulane U Health Sci Ctr, New Orleans; 4) Intl Skeletal Dysplasia Reg, Cedars-Sinai Med Ctr, LA.

This patient had disproportionate short trunk dwarfism, very short chest, midface hypoplasia, diffuse delayed ossification, and flat, ovoid vertebrae. These features were consistent with a spondyloepiphyseal dysplasia (SED), likely spondyloepiphyseal dysplasia congenital (SEDC). Her clinical course was complicated by chronic restrictive pulmonary insufficiency requiring tracheotomy and mechanical ventilation, poor weight gain and recurrent infections, all found in severe SEDC. Beside mild hyperopia, her eyes were normal and palate intact. Atypical features were non-communicating hydrocephalus requiring VP shunt placement and pulmonary hypertension, refractory to all medical therapy including nitric oxide. Despite maximal care, she died at 7 mos. Sequencing COL2A1 revealed a novel 36 bp insertion in exon 48 at cDNA position c.3315. The gly-X-Y amino acid sequence was maintained. Type II collagen is in articular cartilage, spine nucleus pulposus and eye vitreous. COL2A1 produces 1(II) polypeptide chains which form a triple helix homotrimer, twisting from carboxy (C) to amino (N) terminus. COL2A1 mutations are found in SEDs ranging from mild early-onset arthritis, to more severe yet viable Kniest dysplasia, SED Strudwick, and SEDC, to perinatal lethal hypochondrogenesis and achondrogenesis type II. These are dominant negative mutations, with a trend (not absolute) of worsening phenotype as nearing the C terminus. Deletion, splice, duplication, insertion, and most commonly missense mutations (often disrupting gly-X-Y) are described in SEDs. Despite maintaining the gly-X-Y motif, the proximity of our patient's mutation to the C terminus likely explains her severe phenotype. Hydrocephalus and pulmonary hypertension are not described in SEDC. It is possible our patient had one of the lethal SEDs and survived due to extraordinary measures. Perhaps hypochondrogenesis and/or achondrogenesis type II are associated with hydrocephalus and pulmonary hypertension, but unappreciated due to early lethality.

Neonatal Marfan Syndrome: two cases with some unusual clinical findings. *B. Lo¹, G. Maegawa¹, P. Satodia¹, H. Whyte¹, M. Semer¹, J. Hutchinson¹, G. Wilson¹, G. Gross¹, G. Matyas², A. Hinek¹, S. Unger³, D. Chitayat^{1,4}.* 1) Div Clinical Genetics, Hosp Sick Children, Toronto, ON, Canada; 2) Division of Metab and Molec Pediatrics, Univ. Children's Hosp, Zurich, Switzerland; 3) Div of Molec Pediatrics, Univ. Hosp CHUV, Lausanne, Switzerland; 4) Prenatal Diag. and Medical Genetics Program, Mount Sinai Hosp, ON, Canada.

Marfan syndrome is an AD disorder of connective tissue caused by mutations in the fibrillin-1 gene. We report two cases with some unusual clinical finds.. Case1: female born at 37 weeks gestation with loose redundant facial skin, deep-set eyes, arachnodactyly, joint contractures and the hepatic veins connecting to the rt atrium which has not been previously reported in nMFS. A Rt cong. diaphragmatic hernia was diagnosed prenatally at 28 weeks gestation. The baby developed collapse of much of the Lt lung and lower segment of lower Rt. lobe. A missense mut c. 3202T>G on exon 25 of FBN-1 gene was detected. This mut. occurs in a calcium binding epidermal growth factor like domain 11. The fibroblast immunostaining findings showed a disorganization of collagen and elastin and lacking of fibrillin-1 fibers. Case 2: male infant born at term with dolicomacrocephaly, deep set eyes, loose redundant skin over the head and neck, pectus carinatum, marked arachnodactyly, joint contractures, mitral valve regurgitation and dilatation of the aortic and pulmonary roots. The brain MRI revealed interdigitation of the interhemispheric fissure suggesting a partial absence of the falx and a cerebellum displaced upwards which was at first time in nMFS. His neurological examination was normal. At 3 months, the patient died secondary to severe mitral valve insufficiency. A novel G > A transition mut. was identified and it is predicted to affect the invariant +1 position of the splice donor site in intron 30 leading to skipping of exon 30 of the FBN-1 gene. We have two novel mut. in two nMF with some unusual clinical findings such as the brain MRI finds. and the abnormal vascular hepatic vein connection seen in case. It is early to make association of these novel mutation to the these new clinical findings. Further nMFS patients with these muts. should be described.

Expanding the spectrum of phenotypes associated with *COL10A1* mutations - Schmid type of metaphyseal chondrodysplasia with normal height. *O. Mäkitie*^{1,2}, *M. Susic*¹, *W.G. Cole*¹. 1) Research Institute and Division of Orthopaedics, The Hospital for Sick Children, Toronto, ON, Canada; 2) Hospital for Children and Adolescents, University of Helsinki, Helsinki, Finland.

Schmid type of metaphyseal chondrodysplasia (MCDS; MIM# 156500) is an autosomal dominant chondrodysplasia characterized by progressive growth failure resulting in short-limbed short stature (adult height 135-145 cm), and by lower limb deformity which often requires surgical intervention. Radiographic features include widened and irregular growth plates especially in the distal and proximal femora. MCDS results from autosomal dominant mutations in *COL10A1*, the gene encoding for the 1() chains of type X collagen. Type X collagen is a short-chain, non-fibrillar collagen synthesized specifically by hypertrophic chondrocytes at sites of endochondral ossification.

We describe a patient with MCDS and a novel *COL10A1* mutation who had an unusually mild presentation with normal height, late onset of symptoms and lack of lower limb deformity. This female was born at term to healthy parents. Her early growth and development were unremarkable. From age 4 years she was noticed to have a limp and was at age 8 years referred to orthopaedic assessment because of bilateral hip pain and abnormal gait. She was found to have restricted hip movements but no lower limb deformity. Radiographically she had short femoral necks and coxa vara bilaterally; the proximal femoral growth plates showed evidence of mechanical failure and stress fracture. Otherwise her radiographs showed very little metaphyseal involvement. Her height at age 9.6 years was 127 cm (-1.7 SDS). *COL10A1* sequence analysis showed a heterozygous nucleotide substitution 1942G>A which results in a single amino-acid substitution D648N.

Our findings suggest that 1) the clinical variability of *COL10A1* associated skeletal dysplasia is greater than previously thought, and that 2) type X collagen plays a key role in femoral neck development and may be an important determinant of its length, width and neck-shaft angle which have important implications to susceptibility to hip fracture.

Clinical and molecular evaluation of Small Patella Syndrome. *I. McIntosh¹, J. Zaveri¹, R.S. Lachman², J.E. Hoover-Fong¹.* 1) Inst Genetic Medicine & Greenberg Ctr, JHMI, Baltimore, MD; 2) Cedars-Sinai, Los Angeles, CA.

Small patella syndrome (SPS; Scott-Taor syndrome, ischio-pubic-patellar syndrome, ischiopatellar dysplasia) is characterized by patellar hypoplasia/aplasia, infra-acetabular axe-cut notches and abnormal ossification of the ischio-pubic joint. Additional clinical features include a wide gap between toes 1 and 2, pes planus, and short 4th and 5th metatarsals. Heterozygous mutations of TBX4 have been identified in individuals with SPS (AJHG 74:1239). The distinction between similar diagnoses is of crucial importance when the prognoses differ significantly. We describe a family of 3 affected individuals in 2 generations in which the 32 year old female proband presented with the diagnosis of Nail Patella Syndrome (NPS), a distinct disorder associated with patellar aplasia/hypoplasia, but also characteristic nail and elbow anomalies and iliac horns, and a greatly increased risk of glaucoma and nephropathy. NPS was ruled out clinically and by mutation testing of LMX1B. X-ray revealed hypoplastic ischio-pubic synchondroses and bilateral hypoplastic patellae. Physical exam revealed wide space between toes 1 and 2, pes planus, and short 4th and 5th metatarsals. The clinical diagnosis of SPS was assigned and subsequently confirmed via mutation testing of TBX4 with identification of a previously described frameshift mutation. The patient also had a history of depression, anxiety, ADHD and executive dysfunction. Her ophthalmic history is significant for recurrent strabismus, severe myopia, bilateral congenital cataracts and astigmatism. The proband's sister (28 yrs) and niece (3 yrs) also have hypoplastic patellae, an increased gap between toes 1 and 2 and pes planus. Dyslexia, anxiety and a learning disability were also present in the sister. Knee and hip problems, ADHD and other learning disorders were reported in family members unavailable for physical examination. TBX4 is broadly expressed in the developing hindlimb, and is required for normal development of the hindlimb and allantois. Whether the ocular and neuropsychiatric findings observed in the proband described here are coincidental, or represent an extension of the SPS phenotype, remains to be determined.

Discriminant analysis of metacarpophalangeal patterns in multiple genetic conditions. *F.J. Meaney*¹, *M.G. Butler*^{2,3}, *M.F. Davis*¹. 1) Dept of Pediatrics, Univ of Arizona, Tucson, AZ; 2) Section of Medical Genetics & Molecular Medicine, Children's Mercy Hospitals & Clinics, Kansas City, MO; 3) Dept of Pediatrics, Univ of Missouri-Kansas City Sch of Med, Kansas City, MO.

The evaluation of metacarpophalangeal (MCP) patterns in genetic conditions applies a standard anthropometric technique to quantify the amount and direction of abnormal growth in the hand skeleton. The aim of this research was to identify specific multivariate patterns that distinguish several genetic conditions through the application of stepwise discriminant analysis. Measurements of the hand bones were made from radiographs on 96 individuals who had one of five conditions and forty-one healthy controls. The five conditions included Fragile X syndrome (n = 18), Noonan syndrome (n = 15), Prader-Willi syndrome (n = 31), Sotos syndrome (n = 16) and Diastrophic dwarfism (n = 16). More than one clinical geneticist diagnosed each affected individual. The age range of the total sample was from less than 1 month to 40 years, with a mean of 11.5 years (SD 8.4). Z score values were computed for the nineteen hand bones in each individual using published bone length standards. The Z score data and age were used in the stepwise discriminant analysis. Eleven of the 19 MCP variables, but not age, were included in the four functions that differentiated the groups. The analysis produced a correct classification rate of 88%. The first two functions accounted for almost 84% of the variance among groups. The first function describes overall hand bone size, and separates individuals with Diastrophic dwarfism and Sotos syndrome at the extremes. The second function separates individuals with Prader-Willi syndrome from the other five groups, with distal and middle phalanges of the 4th and 5th fingers producing the highest correlations with scores on this function. These data demonstrate the utility of discriminant analysis in the description of specific patterns in hand bone growth among several genetic conditions and its continuing application as a diagnostic tool using the MCP data.

Targeted Molecular Testing Reveals a Microdeletion in a Child with Classic Genetic Disorder and Additional Multi-System Involvement. *L. Medne¹, J.D. Hoffman¹, I. Krantz¹, R. Mendoza-Londono², D. Napierala^{2,3}, P. Stankiewicz², B. Lee^{2,3}, E.H. Zackai¹.* 1) Division of Human Genetics, Children's Hospital of Philadelphia; 2) Department of Human and Molecular Genetics, Baylor College of Medicine; 3) Howard Hughes Medical Institute.

We report a 10 y/o male with intra uterine growth retardation, microcephaly, cardiac ventricular septal defect, severe short stature, micropenis, hypospadias, and moderate MR. He had had very large fontanelles, hypertelorism, flat nasal bridge, brachydactyly, and delayed eruption of teeth. Chest X-rays showed hypoplastic clavicles, abnormal ribs and lack of sternal ossification, strongly suggesting a diagnosis of cleidocranial dysplasia (CCD). Chromosome analysis was normal. Molecular analysis of the coding region and the promoter region of the RUNX2 gene did not reveal any mutations. We predicted that this child with classic CCD findings, MR and multi-system involvement could have a microdeletion. FISH analysis with three BACs, which contain segments of the RUNX2 gene and map to 6p21, confirmed the presence of a microdeletion encompassing the RUNX2 gene. Preliminary studies suggest that the deletion is approximately 3Mb in length. Breakpoints of the deleted segment are being defined. Mundlos et al (1997) and Lee et al. (1997) showed that heterozygous mutations in the RUNX2 gene cause CCD and that most affected individuals have an intragenic mutation. Visible chromosomal translocations/inversions led to the identification of the gene but are a rare cause of CCD, with 4 reported cases in the literature. There are 2 cases of classic CCD with a cryptic microdeletion of 1.5 Mb in one family (Mundlos et al., 1997), and of unknown length in another (Zhang, 2002). A similar approach has been used in the past to explain other expanded phenotypes such as the NF1 plussyndrome with 17q11.2 deletions encompassing NF1 gene (Leppig et al., 1997) and Saethre-Chotzen plus phenotype with 7p21.1 deletions encompassing TWIST gene (Johnson et al., 1998). We recommend, that in a patient with a classic syndrome and additional systemic findings, a cryptic microdeletion should be looked for with targeted molecular testing.

RADIOLOGIC MANIFESTATIONS OF 5 BRAZILIAN CASES OF MUCOPOLYSACCHARIDOSIS VI (MAROTEAU-X-LAMY SYNDROME). *A.C. Paula¹, A.C.G.M. Santos¹, T.P. Delboni¹, D.R. Bertola¹, L.M.J. Albano¹, L.A.N. Oliveira², R. Giugliani³, C.A. Kim¹.* 1) Pediatrics, Instituto da Crianca- HCFM-USP, So Paulo, Sao Paulo, Brazil; 2) Radiology, Instituto da Crianca- HCFM-USP, So Paulo, Sao Paulo, Brazil; 3) Servico Genetica de Porto Alegre- HC-UFRGS, Brazil.

Mucopolysaccharidosis type VI (MPS VI), also known as Maroteaux-Lamy syndrome, is an autosomal recessive lysosomal storage disorder caused by a hydrolase deficiency - the N-acetylgalactosamine-4-sulfatase - formerly known as arylsulfatase B, that is responsible for dermatan sulfate degradation. There are three recognized clinical entities: mild, moderate and severe. The course is progressive involving multiple systems, specially skeletal, cardiac and pulmonary ones. We report on 5 Brazilian patients with MPS VI. Radiologic findings were: 1) Skull: macrocephaly and premature closure of the sutures (100%); olympic forehead (80%); thick calvaria, underdevelopment of mastoid cells, vascular impressions, large omega-shaped sella turcica(60%); 2) Thorax: canoe paddle appearance of the ribs and hypoplastic glenoid fossae (100%); thick clavicles and elevated scapulae (80%); 3) Vertebrae: platyspondyly (80%); 4) Long bones: hatchet-shaped proximal portions of the humeri (100%), tilt of the distal parts of the radius and ulna toward, thickened cortex with prominent bone trabeculation epiphyseal (80%) and metaphyseal (60%) irregularities, enlarged diaphyses, and lacunar lesions (60%); 5) Pelvis and hips: irregularity and underdevelopment of the acetabular roofs (100%), flared small iliac wings, thick ischial and small femoral heads, fragmentation of the femoral head (80%); 6) Hands and feet: small and irregular carpal bones (100%); shortened phalanges (80%); widening of of metacarpal diaphyses (60%). Conclusion: Radiographic findings were consistent of a multiplex dysostosis, typical of MPS type VI, and the more severe skeletal abnormalities were observed in older patients.

Reduced bone mineral content in Marfan syndrome patients. *K. Phan¹, N. Brunetti-Pierri¹, S. Carter^{1,2}, J.W. Belmont¹, J.A. Towbin³, K.J. Ellis⁴, B. Lee^{1,2}.* 1) Department of Mol & Hum Genet, Baylor College of Medicine, Houston, TX; 2) Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX; 3) Department of Pediatrics, Section of Cardiology, Baylor College of Medicine, Houston, TX; 4) Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX.

Marfan syndrome is an autosomal dominant disorder of connective tissue characterized by defects of cardiovascular systems, eye, and skeleton. Skeletal findings include tall stature, arachnodactyly, and vertebral defects. The presence of osteoporosis/osteopenia in Marfan syndrome is uncertain and its occurrence is still debated, especially in pediatric patients. In Marfan syndrome the bone mineral content (BMC) provide a more accurate measurement of bone status than bone mineral density (BMD). In a cross-sectional study we have assessed bone mineral status of children and adolescents with Marfan syndrome by dual energy X-ray absorbiometry (DXA). The cohort included 14 males and 5 females [mean age: 9.54.8 yrs] seen in a cardiology clinic. The mean whole body BMC z-score was significantly reduced [-1.691.41] ($p < .001$), while the BMD z score at lumbar spine and other regional sites resulted to be normal. 47% (9/19) of the patients had a z-score in the osteopenia/osteoporosis range with 21% (4/19) of patients in the osteopenic range (z-score < -1.5), and 26% (5/19) in the osteoporosis range (z-score < -2.5). Because individuals gain much of their peak bone mass by the end of adolescence, those not attaining optimal amounts during growth are at risk for the development of osteoporosis and fractures later in life. Therefore the early detection of Marfan syndrome children with low BMC is clinically important for prevention program designed to improve bone status and prevent long term consequence of osteoporosis. Marfan syndrome is caused by loss-of-function mutations in the gene encoding fibrillin-1, which has recently been shown to regulate TGF- activation. Interestingly, TGF- is an important regulator of both skeletal development and homeostasis of bone metabolism and polymorphisms in TGF- gene are a risk factor for osteoporosis.

Severe spondyloepimetaphyseal dysplasia with profound mental retardation : a new autosomal recessive

MCA/MR syndrome. *P. Sarda¹, N. Bigi¹, J. Puechberty¹, C. Coubes¹, G. Lefort², P. Blanchet¹.* 1) Department of Medical Genetics, Hopital Arnaud de Villeneuve, CHU de Montpellier, 34295 Montpellier, France; 2) Department of Medical Genetics, Cytogenetics Laboratory, Hopital Arnaud de Villeneuve, CHU de Montpellier, 34295 Montpellier, France.

We report four sibs born of non consanguinous parents and presenting an unusual syndrome including severe spondyloepimetaphyseal dysplasia and profound mental retardation. After the birth of a normal boy, the couple's second pregnancy with monozygotic female twins was terminated at 34WG because hydrocephaly and cerebral calcifications were detected in both fetuses. One fetus presented flat dysplastic acetabulae on skeletal X-rays. Subsequently, the third pregnancy was also terminated at 25WG because of corpus callosum agenesis and ventriculomegaly in a male fetus. Flat dysplastic acetabulae were also present on skeletal X-rays. The couple's fourth pregnancy was normal. At birth, the apparently healthy boy had a normal cerebral ultrasound. Hypotonia and psychomotor retardation developed at 7 months. Chromosome studies and metabolic tests were normal. The child presented severe osteochondrodysplasia evidenced on X-rays which showed platyspondyly, dysplastic iliae with a lacey border of iliac crests, irregular metaphysis, extreme brachydactyly and highly dysplastic carpal and tarsal bones. Cerebral MRI revealed cerebral atrophy with ventriculomegaly. At the age of 6 years, there is severe mental retardation with no language and total hypotonia. In addition, the boy has convulsions and required tracheotomy because of major respiratory distress due to impaired swallowing mechanism.

Skeletal abnormalities, dysmorphic features and developmental delay in siblings: A new autosomal recessive syndrome? *R.L. Sparkes, D.R. McLeod.* Medical Genetics, Alberta Children's Hospital, Calgary, Alberta, Canada.

We report a boy and girl born to consanguineous Pakistani parents with skeletal abnormalities, dysmorphic facies, and mild to moderate developmental delays. Both parents and four other siblings were normal. Cranial abnormalities in the affected sibs included sagittal and lambdoidal synostosis with dolichocephaly, frontal bossing and midline defects of the posterior occiput. Skeletal abnormalities included bilateral midclavicular pseudoarthroses and widening of the lumbosacral canal, with normal stature. Dysmorphic facial features included hypertelorism, ptosis, high-arched palate and oligodontia. Other clinical findings were bilateral hearing loss, paresis of the inferior rectus muscles which depress the eyes, unusual fibrotic skin lesions, and recurrent pancreatitis. Genetic and metabolic investigations, including karyotype, transferrin isoelectric focusing, subtelomeric FISH, and plasma and urine amino and organic acids were all normal. Extensive review of the dysmorphology literature failed to reveal any similar phenotypes. Although this syndrome shares some features with cleidocranial dysplasia and Saethre-Chotzen syndrome, the latter are autosomal dominant conditions. It is the authors' opinion that these siblings have a rare autosomal recessive condition that has not been previously described.

Bellini metaphyseal dysplasia: Two unrelated cases highlight variability in extra-skeletal manifestations. *V.R.*

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Bellini Metaphyseal Dysplasia (BMD), also known as Metaphyseal Acroscyphodysplasia (OMIM 250215), is a rare skeletal dysplasia with unusual cone-shaped metaphyses of the distal femora. Most published cases seem to have a variety of non-skeletal manifestations. We describe two unrelated patients with BMD and various extra-skeletal abnormalities. A 26-month-old Caucasian female was evaluated during the neonatal period because of dysmorphic features; At 18 months she was noted to have bilateral limitation of knee extension. At 26 months her weight was 12.5 kg (25-50%) and height 82 cm (5%); she has hypotelorism, narrow & flat nasal root, shallow orbits, nystagmus and a single upper central incisor. There is bilateral limitation to extension of the knees. Skeletal survey showed deformity of the distal femoral and proximal tibial metaphyses, characteristic of BMD. A brain MRI was normal. Chromosome analysis was normal as was FISH for seven holoprosencephaly loci and other microdeletions. The other is a 9.5-year-old black male seen because of leg length discrepancy, and found to have developmental delay, a seizure disorder and precocious puberty. His weight was 29 kg (25%), and height 120.6 cm (<5%). Extremities showed rhizomelic shortening and an 18 cm leg length discrepancy. Skeletal survey revealed cone-shaped metaphyses of the distal femora bilaterally, but was otherwise normal. Endocrinologic testing indicated a central origin of precocious puberty; a brain MRI was normal. The first case of BMD was described in 1966 and since then only a handful of patients with this autosomal recessive metaphyseal dysplasia have been reported. Common features are short stature, MR, accelerated bone age and a knee metaphyseal dysplasia. Based on the variability in non-skeletal manifestations we hypothesize that BMD is a microdeletion disorder involving a gene for skeletal development - most likely related to chondrocyte differentiation.

Analysis of skeletal phenotype and exclusion of mutation in BMP7 gene in two cases of short rib polydactyly syndrome type Majewski. *C.T. Thiel¹, A. Dimmler², H. Stoess³, M. Zenker¹, E. Beinder⁴, T. Aigner², A. Reis¹, A. Rauch¹.* 1) Institute of Human Genetics, University of Erlangen-Nuremberg, Germany; 2) Institute of Pathology, University of Erlangen-Nuremberg, Germany; 3) Institute of Pathology at St. Johannisstift, Paderborn, Germany; 4) University Hospital of Gynaecology and Obstetrics, University of Erlangen-Nuremberg, Germany.

The short-rib-polydactyly syndromes (SRPSs) are a heterogenous group of skeletal dysplasias characterized by short-limb dysplasia, narrow hypoplastic thorax with short ribs, polydactyly and associated visceral abnormalities, mainly polycystic kidneys. To date a responsible mutation in a defined gene could be identified only for the Ellis-van-Creveld syndrome (EVC). This EVC gene was excluded as causative for other SRPS, as demonstrated by linkage analysis. In order to further elucidate the genetic background of SRPSs we compared phenotypic features of affected individuals with developmental defects of knockout models in mice. Previous studies analyzed the histological changes in endochondral ossification in SRPS, mentioning mainly a disorganization and irregularity of the growth plate in long bones. Our studie showed that changes in endochondral ossification represent a partly arrested orthotopic ossification at the stage of the hypertrophic chondroblast and heterotopic perichondral ossification with accessory ossicles in the lower leg of one case. Impaired renal development is indicated by polycystic kidney disease with dilated collecting tubules and glomerulocystic changes in the other case. Excessive polysyndactyly and median cleft palate was apparent in both cases. Due to apparent phenotypic analogies to the knockout mouse model for BMP-7 we excluded impaired expression or mutation of this morphogen as a causative factor for SRPS in the two clinical cases by immunohistochemical methods and analysis of genomic sequence.

A new syndrome of congenital generalized osteosclerosis and bilateral polymicrogyria. *L. Titomanlio¹, G. Bonyhay², Y. Hutten², J.F. Oury³, E. Vuillard³, C. Garet⁴, P. Terdjman⁴, C. Baumann¹, A. Verloes¹, A.L. Delezöide⁴.* 1) Clinical Genetics Unit; 2) Fetal pathology Unit; 3) Obstetric department; 4) Medical Imaging department, Robert Debré Hospital, Paris, France.

We report on a 29 weeks male fetus born to consanguineous healthy parents. He showed a severe sclerosing bone disorder affecting all skeletal elements (membranous bones, face, long bones, axial skeleton), resulting in insufficient modelling, generalized densification and fragility of the skeleton. The basis of the skull and facial bones were extremely sclerotic and mandibular dental germs were not visible. Long bones (including extremities) and spine were irregularly shaped with insufficient metaphyseal wedging. Diaphyseal bones were extremely dense, with thick cortex and absent medullar canal. The first metacarpal and the distal phalanges were short, the latter with terminal tufting. This skeletal dysplasia was associated with an abnormal craniofacial development, consisting of hypertelorism, severe microretrognathia, cleft palate, absent epiglottis, and reduced number and delayed mineralization of teeth buds. Epiphyseal cartilage and growth plate had a normal architecture and cellularity. Cortical bone was thin and irregularly lined. Endostal canals were unusually narrow. Cancellous bone counted numerous osteoblasts and was less compact than normally seen. The matrix was irregular and insufficiently mineralized. Remodelling activity was reduced, and the medullar cavity was not developed. Neuropathologic examination showed a corpus callosum that lacked genu and splenium and bilateral symmetric fronto-parietal cerebral polymicrogyria. This syndrome of unknown origin appears to represent a new variant within the congenital sclerotic bone disorders family. Autosomal recessive inheritance is possible.

Osteoporosis, severe mental retardation, microcephaly and epilepsy : a new syndrome. C. Vinkler^{1,2}, T. Lermansagie², M. Yanouv-Sharav^{1,2}, M. Ginzberg², N. Watemala², O. Vardi², D. Lev^{1,2}. 1) Inst Medical Genetics, Wolfson Medical Ctr, Holon, Israel; 2) Metabolic Neurogenetic Clinic Wolfson Medical Ctr, Holon, Israel.

Bone fragility has been previously described in some sporadic and rare syndromes. We report a girl with a unique combination of severe osteoporosis, dysmorphic features and neurological symptoms. This 12 years old girl was born to healthy unrelated parents, following an uneventful pregnancy. After her birth, small head circumference and dysmorphic features were noticed. At the age of one month she had tonic seizures. She still has epileptic seizures which are controlled to a large extent by a combined anticonvulsive therapy. Serial brain MRI scans revealed partial agenesis of corpus callosum and progressive brain atrophy. Severe osteopenia was already noticed at the age of nine months with hypercalciuria and nephrolithiasis. Lab tests did not reveal abnormal PTH or Vit D metabolism. Based on skeletal survey, laboratory tests and the early clinical presentation it is less likely that bone fragility is due to disuse, although her complete immobilization along with the long-term use of anticonvulsants added to the severe osteoporosis. She was started on pamidronate treatment for her severe osteopenia. At the age of 12 years she has severe mental retardation. Her weight, height and head circumference were -4 S.D. She is not ambulatory and does not speak. She has good eye contact with her family and her caretakers. Hearing is normal. Extensive evaluation including metabolic work-up, chromosome analysis, telomere screening and molecular studies to rule out fragile X syndrome and Angelmann syndrome were all normal. Differential diagnosis includes: Cole-Carpenter syndrome with craniosynostosis, OI like syndrome with extrapyramidal signs, osteoporosis-pseudoglioma syndrome, bone fragility and retinopathy syndrome, sparse hair and mental retardation, brittle bone and leukodystrophy and in bone fragility, congenital cataract and microcephaly syndrome. However our patients phenotype differs from the previously described syndromes. We suggest that this girl has a previously undescribed syndrome.

Hypophosphatasia and homozygosity for a paternal heterozygous G403S TNSALP mutation due to segmental paternal isodisomy for chromosome 1p. *D. Wand¹, C. Glaeser¹, I. Brun-Heath², H. Thiele¹, A. Herrmann³, A. Musil⁴, I. Hansmann¹, E. Morner².* 1) Inst. of Human Genetics, Univ. Halle, Halle, Germany; 2) Centre d'Etudes de Biologie Prenatale, SESEP, Versailles, France; 3) Klinik und Poliklinik f. Geburtshilfe und Reproduktionsmedizin, Univ. Halle, Germany; 4) Institut f. Pathologie, Univ. Halle, Germany.

Hypophosphatasia is an inherited disorder characterised by defective bone and teeth mineralization and deficiency of serum and bone alkaline phosphatase activity. Symptoms are highly variable; severe perinatal and infantile forms (HOPS, OMIM 241500) are transmitted as an autosomal recessive form while milder forms follow both autosomal recessive and dominant mode of inheritance. A wide spectrum of mutations within the TNSALP gene at 1p36.1 have been described in severe and in mild forms. Compound heterozygous missense mutations account for most of the severe cases. We report about a severe form detected sonographically at week 19. After termination the male fetus showed skeletal anomalies of a metaphyseal type of chondrodysplasia compatible with hypophosphatasia. Direct sequencing of the TNSALP coding sequence including exon-intron borders and untranslated exons using chorionic villus DNA identified a 1258 G to A mutation (G403S). Sequencing exon 11 revealed heterozygosity for G403S in the father with hypomineralisation of teeth but no mutation in the clinically normal mother. Genotyping both parents and fetus for 20 microsatellites spanning a 205.5cM chromosome 1 segment between D1S228 (1p36.21) and D1S439 (1q42.12) uncovered lack of maternal alleles and the presence of only one paternal allele for all 1p loci distal to D1S2753 (1p21.3, 120cM) but heterozygosity and biparental inheritance for all 1q and 1p21 markers. Karyotyping at 450 G-band level and FISH using probe for 1p36 did not reveal any evidence for a deletion. It is concluded that the fetus is homozygous for the paternal G403S mutation due to paternal isodisomy for an at least 32.7Mb 1p-segment.

Further delineation of Majewski/ hydrolethalus syndrome in an Old Order Amish kindred. *S. Bakker², V.M. Siu^{1,2,3}*. 1) Department of Pediatrics; 2) University of Western Ontario; 3) Medical Genetics Program of Southwestern Ontario, London Health Sciences Centre, London, ON, Canada.

Short rib-polydactyly syndrome II (Majewski syndrome) and hydrolethalus are lethal autosomal recessive conditions which share features of polydactyly, shortened limbs, median cleft lip and palate, deep-set eyes and a lobulated tongue. Cardiac anomalies, pulmonary hypoplasia, abnormal genitalia, and polyhydramnios may occur. Hydrolethalus exhibits severe hydrocephalus and agenesis of the corpus callosum. Majewski syndrome characteristically shows shortened, ovoid tibias. Hypoplastic epiglottis has been described in Majewski syndrome but not hydrolethalus. We describe two Old Order Amish brothers and their male first cousin who exhibited features of both syndromes. The elder brother, stillborn at 34 weeks to second cousin parents, exhibited severe progressive hydrocephalus, short limbs, and narrow chest on prenatal ultrasound. Postaxial polydactyly and synbrachydactyly were noted on all limbs. Karyotype was 46, XY but external genitalia appeared female. Median cleft lip and palate were present. The younger brother, induced at 24 weeks due to multiple anomalies on ultrasound, exhibited similar features. Autopsy revealed microphthalmia with cataracts and retinal dysplasia, rudimentary sulcation of the brain, and agenesis of the corpus callosum. There was disordered endochondral ossification. X-rays did not demonstrate short ribs or ovoid tibias. Tongue was lobulated. Hypoplastic epiglottis and small lungs were noted. Adrenals were absent. A paternal first cousin to the brothers was born at 33 weeks with similar features and died at 4 days. The parents were also consanguineous. We believe that these three cases offer further evidence that Majewski syndrome and hydrolethalus may represent entities along a continuum resulting from a single genetic defect, and add absent adrenals and sex reversal as further features.

Longitudinal vestibular schwannoma growth rates in neurofibromatosis 2 (NF2): a combined analysis from three countries. *M.E. Baser*¹, *T. Patankar*², *L. Kluwe*³, *V.-F. Mautner*⁴, *E. Makariou*⁵, *D.M. Parry*⁶, *A.J. Wallace*⁷, *R.T. Ramsden*⁸, *D.G.R. Evans*⁷, *A. Jackson*². 1) Los Angeles, CA, USA; 2) Division of Imaging Science and Biomedical Engineering, University of Manchester, Manchester, UK; 3) Department of Neurosurgery, University Hospital Eppendorf, Hamburg, Germany; 4) Department of Neurology, Klinikum Nord Ochsensoll, Hamburg, Germany; 5) Department of Radiology, Georgetown University School of Medicine, Washington, DC, USA; 6) Genetic Epidemiology Branch, National Cancer Institute, Bethesda, MD, USA; 7) University Department of Medical Genetics, St. Mary's Hospital, Manchester, UK; 8) Department of Otolaryngology, Manchester Royal Infirmary, Manchester, UK.

We previously found that vestibular schwannoma (VS) growth rates in NF2 tended to decrease with increasing age and that genotype-phenotype correlations were not apparent. To more definitively address these questions, we assessed VS growth rates in 84 NF2 patients from the United States, Germany, and the United Kingdom. The median follow-up was 3.9 years (range, 0.2 to 14.8 years). Box models were used to estimate VS volumes and linear regression was used to evaluate the association between tumor doubling time (TDT, in years) and covariates. VS growth rates decreased with increasing age at baseline ($\log_{10} \text{TDT} = .834 + .008 \times \text{age at baseline}$, $\text{SE}_b = .004$); people aged ≤ 15 years at baseline had the highest growth rates. Genotype-phenotype correlations were not apparent. To estimate the effect of volumetric accuracy on growth rates, we used two methods (one- and two-component box models) to estimate TDT for 26 VSs that had intracanalicular and extracanalicular portions; the volumes of tumors with two portions are measured more accurately in the two-component model. One-component model TDTs were less than two-component model TDTs by a median of 12%. These results suggest that different three-dimensional VS volumetric methods have a relatively small effect on average VS growth rates, possibly because the magnitude of long-term longitudinal VS volume change is often far greater than the magnitude of VS volume measurement error.

Oculofaciocardiodental and Lenz microphthalmia syndromes result from mutations in the X-linked transcriptional corepressor, BCOR. *G. Black¹, D. Ng², C. Corcoran³, D. Donnai¹, S. Burgess⁴, N. Thakker¹, V. Bardwell³, L. Biesecker².* 1) Dept Clinical Genetics, St Mary's Hosp, Manchester, United Kingdom; 2) Genetic Disease Research Branch, National Institutes of Health, Bethesda, MD, USA; 3) Department of Genetics, Cell Biology and Development/Cancer Center, University of Minnesota, Minneapolis, MN, USA; 4) Genome Technology Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA.

Lenz microphthalmia syndrome comprises microphthalmia with mental retardation, skeletal and other anomalies. Inherited in an X-linked recessive pattern, there are putative loci at Xq27-q28 (MAA) and Xp11.4-p21.2 (MAA2). After refining the MAA2 locus, sequencing of candidate genes showed a substitution c.254C>T, p.P85L, in BCOR (BCL-6 interacting corepressor) in affected males from a Lenz syndrome family. Oculo-facial-cardio-dental syndrome (OFCD) is inherited in an X-linked dominant pattern with presumed male lethality. It is characterized by microphthalmia, dysmorphic facies, radiculomegaly, cardiac and digital abnormalities. Given their phenotypic overlap, we hypothesized that OFCD and MAA2 were allelic and found different frameshift, deletion and nonsense mutations in BCOR in OFCD families. Functional studies of wild type and p.P85L mutant BCOR suggest that BCOR interacts with other transcription factors and perturbation of this interaction causes these two syndromes. Knock down expression of the zebrafish BCOR ortholog, which shows 40% amino acid identity to the human protein, caused developmental perturbations of the eye, developing skeleton, and CNS, consistent with the human syndrome and confirming that BCOR is a key transcriptional regulator during early embryogenesis.

APLASIA CUTIS CONGENITA, SKULL DEFECT, BRAIN HETEROTOPIA, AND INTESTINAL

LYMPHANGIECTASIA. *E.V. Bonioli¹, R.C. Hennekam², C. Bellini¹*. 1) Pediatrics Department, Gaslini Institute, University, Genoa, Genoa, Italy; 2) Departments of Pediatrics and Clinical Genetics, University of Amsterdam, The Netherlands.

This girl was born at term to healthy, non-consanguineous parents following an uneventful pregnancy. During pregnancy a congenital defect of the scalp involving the underlying bone (aplasia squama frontalis) had been demonstrated. We investigated the patient at the age of 7 months. She presented an oval area (6 cm diameter) of healing non-inflamed, well demarcated, epithelized, with no blister skin covering a bony scalp defect. Irregular hair growth around the scalp lesion was present. She showed a depressed nasal bridge, synophrys, telecanthi, hypertelorism, and long philtrum. Peripheral lymphedema was evident (lower leg). High fecal excretion of alpha-1-antitrypsin suggested intestinal lymphangiectasia. Lymphoscintigraphy (age 7 months) showed initial dermal back-flow in the left foot, indicating an accumulation of the radiocolloid in the dilated channels of the dermis. Inguinal lymph nodes were delayed observed. Three-dimensional CT scan (age 7 months) demonstrated aplasia of the frontal bone, widely patent cranial sutures, and subtotal reconstitution of the frontal bone. Sagittal T2-weighted MR image (age 1 month) showed subcortical frontal heterotopia. A normal psychomotor development and a stationary degree of peripheral lymphedema is evident (age 12 months). In conclusion, we describe a patient with an unusual combination of symptoms including aplasia cutis congenita with underlying skull defect at an unusual locus, cortical dysplasia, congenital peripheral lymphedema, and intestinal lymphangiectasia. Some of the characteristics would fit the OMIM 207731 entity, others fit a RELN mutation (OMIM 600514), and several symptoms are also found in the OMIM 235511. Next to these entities a disruption sequence initiated by a lymph vessel dysplasia and thereafter involving brain and skin might also be an explanation. A last possibility might be the presence of a separate, hitherto undescribed entity. Reports on other cases, and detailed evaluation of cases of aplasia cutis for lymphatic vessel dysplasia are needed to allow a more firm conclusion about the pathogenesis.

Polymorphism within the coding region of the LIS1 gene : implications for diagnostic counselling. C. Cardoso¹, S. Das², F. Moro³, A. Moncla⁴, L. Villard¹, J. Mancini⁵, R. Guerrini³, N. Philip ^{*4}, W.B. Dobyns ^{*2}. 1) INSERM U491, Faculte de Medecine la Timone, Marseille Cedex, France; 2) Department of Human Genetics, The University of Chicago, Chicago, IL; 3) Neurogenetics Laboratory, Institute of Child Neurology and Psychiatry, IRCCS Fondazione Stella Maris, Calambrone, Italy; 4) Departement de Genetique Medicale, Hopital des enfants de la Timone, Marseille, France; 5) Departement de Neurologie pediatrique, Hopital des enfants de la Timone, Marseille, France.

Lissencephaly (LIS) is a neuronal migration disorder resulting in a thickened cortex and reduced gyration usually causing severe mental retardation, intractable epilepsy and cerebral palsy. LIS is one component of the contiguous gene deletion disorder known as Miller-Dieker syndrome (MIM:247200) or may occur as an isolated brain malformation in patients with isolated lissencephaly sequence (ILS). The clinical severity generally correlates with the degree of agyria and cortical thickening. Approximately 70 % of patients show abnormalities of the LIS1 gene on 17p13.3 or of the DCX gene on Xq22.3. A more severe lissencephaly in posterior brain regions is generally observed in patients with LIS1 mutations. To date, among all mutations reported in the LIS1 gene only 6 patients have a missense mutation. In the course of screening for LIS1 mutation in ILS patients, we identified by direct sequencing 8 new missense mutations (L51S, K64N, D129V, C281R, G314V, S355N, V364I, D401H). Our data support the hypothesis that, in most cases, the lissencephaly phenotype associated with LIS1 missense mutations is generally milder. However, among these, one (V364I), identified in a ILS boy, was also found in his mother who is mildly mentally retarded but with a normal MRI scan and his grandfather and aunt who are normal thus ruling out its involvement in the etiology of the phenotype of this patient. DCX was sequenced but no mutation was found in this family .These findings clearly call for a careful consideration of the pathogenicity of the LIS1 missense mutations identified in sporadic ILS patients before genetic counselling or prenatal diagnosis is proposed to the corresponding families.*LIS1*.

Depressive symptoms in Machado-Joseph Disease (SCA3) patients and relatives. *C.R. Cecchin¹, A.P. Pires², C.R.M. Rieder³, T.L. Monte³, M.L. Pereira^{1,5}, I. Silveira⁶, T. Carvalho¹, J. Sequeiros⁶, L.B. Jardim^{1,4}.* 1) Medical Genetics Service, Hospital Clinicas Porto Alegre, Porto Alegre, Brazil; 2) Psychology Service, HCPA, Porto Alegre, Brazil; 3) Neurology Service, HCPA, Brazil; 4) Department of Internal Medicine, Universidade federal do Rio Grande do Sul, Brazil; 5) Biochemistry Department, UFRGS, Brazil; 6) UnIGENE, Instituto de Biologia Molecular e Celular, Porto, Portugal.

Objectives: to determine the depression scores of Machado-Joseph disease (MJD) patients, of their spouses, and of individuals at 50% risk for MJD; and to look for some correlation between depressive manifestations and the degree of motor incapacitation. **Methods:** 246 adult individuals were studied: 79 MJD patients (group 1), 43 spouses (group 2) of MJD patients, 80 individuals at risk for MJD (group 3), and a control group of 44 patients with multiple sclerosis (group 4). Two instruments were applied: Beck Depression Inventory (BDI), to quantify the depressive symptoms, and the Barthel index of physical incapacitation (10), both in an adapted version to Portuguese language. **Results:** in the MJD families, the higher average of BDI scores were found among patients, and showed a linear reduction among other family member: they were of 15.6 in group 1, of 10.5 in group 2, and of 5.6 in group 3 ($p < 0.0001$, Anova). Moderate to severe depressive scores were found in 33.5% of MJD patients, and in 16.2% of their spouses. Depressive scores were also associated with age, with female sex and with poor educational level. A direct correlation between BDI scores and motor incapacitation was found among MJD patients ($r = 0.507$, Pearson correlation, $p < 0.0001$). Although depressive scores among the control group of MS were higher than those found in MJD, in MS they did not correlate with physical incapacitation, or with age, or educational level. **Conclusions:** depressive symptoms were rather common in MJD patients, as well as in their caretakers. We suggest that depression is more reactive than primarily related to disease process, in MJD.

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PARTIAL CEREBELLAR HYPOPLASIA IN A PATIENT WITH PRADER-WILLI SYNDROME. *D. De Brasi*¹, *L. Titomanlio*¹, *V. Barletta*¹, *A. Romano*¹, *R. Genesio*², *F. Maio*¹, *D. Melis*¹, *F. Fabbrini*², *A. Conti*², *P. Tedeschi*², *G. Andria*¹, *G. Sebastio*¹, *E. Del Giudice*¹. 1) Dept of Pediatrics, Federico II University, Naples, Italy; 2) Dept of Cellular and Molecular Biology and Pathology, Federico II University, Naples, Italy.

Prader-Willi syndrome (PWS) is a recognized genetic condition due to an imprinting defect of the PWS critical region on 15q11-13. The pathogenetic mechanism is represented by *de novo* interstitial deletion of 15q11-q13 of paternal origin. We recently observed a 2-year-old male infant because of craniosynostosis. Antenatal reduced fetal movements were noted. Moderate hypotonia and feeding problems occurred in the first months. Physical examination at 2.6 years revealed: height 89.5 cm (10-25th centile), weight 17.500 g (>95th centile), OFC 47.5 cm (25-50th centile); facial dysmorphism (overfolding auricle, pointed nose, strabismus), scoliosis and bilateral cryptorchidism. Brain MRI disclosed a right cerebellar hemisphere hypoplasia. His general development quotient was 74 (Griffiths Mental Development Scales). HRB (650 bands) unraveled a 15q interstitial deletion. FISH analysis by using polymorphic 15q microsatellites (D15S63, D15S674 in 15q11) and specific 15q markers (RP11-20B10 and RP11-570N16 in 15q12, and RP11-37J3 and RP11-25D7 in 15q13.1) allowed to restrict the deleted region to 15q11-q12. This cytogenetic aberration was not present in both parents. The PWS methylation test disclosed the absence of paternal contribution. Our patient represent one of the few reported cases of PWS with structural CNS abnormality. Particularly, in some autopsy cases of PWS, cerebellar lesions have been reported (Hayashi et al, 1992)¹. So, it is possible that cerebellar hemisphere hypoplasia is consequential to the deletion. Among the genes mapping to PWS critical region, NECDIN (a paternally imprinted gene, strongly involved in CNS development) could be considered to contribute to the pathogenesis of this cerebellar malformation. Accurate study of the genes localized within the deleted region could help us to unravel this peculiar issue.¹[Brain Dev 1992 14(1):58-62].

Clinical and molecular studies in Spanish Autistic patients. *B. Gener¹, I. Cusco¹, M. Del Campo¹, E. Gonzalez², L. Sumoy², M. Vilella³, A. Infante³, J.A. Munoz⁴, M. Salido⁴, O. Villa⁴, L.A. Perez Jurado¹.* 1) Genetics Unit, Dept. of Experimental Sciences, Universitat Pompeu Fabra, Barcelona, Spain; 2) Microarray Laboratory, Center for Genomic Regulation, Barcelona, Spain; 3) Group Pere Mata, Reus, Spain; 4) Cytogenetics laboratory and Pediatrics Department, Hospital del Mar, Barcelona, Spain.

Autistic disorder (AD) is a complex neuropsychiatric disorder of unknown etiology, although 10-15% of cases have known genetic disorders or chromosomal anomalies. Epidemiological, sibling pair and family studies suggest that autism is a heritable oligogenic disorder, although recurrent genomic mutations might underlie a significant proportion of cases. Genome-wide scans show that several chromosomal regions are involved in the etiology of AD. We are studying patients with AD from two different sources: 22 children followed in Neurology clinic and 59 institutionalized adults. A full clinical characterization aimed at identifying comorbid clinical conditions and a thorough battery of neuropsychological tests are being performed. Major or minor congenital anomalies are present in at least 12 patients, including VATER defects, Down syndrome and Tuberous Sclerosis. Undiagnosed PKU and SCID are present in two patients. G-banding karyotypes and Fragile X studies are indicated based on clinical findings. A 47, XY, +mar. ish invdup (15)(q10;q13) [15] karyotype was found in a classical patient with AD and mild dysmorphic features. is being to all patients. We have developed several new molecular tools aimed at identifying recurrent genomic mutations: 1) A multiplex quantitative real time PCR assay with TaqMan probes to simultaneously detect potential duplications at 15q11.2, 17p11, and 22q11.2 regions; 2) a microarray-based Comparative Genomic Hybridation (aCGH) assay containing 216 BAC for single copy regions flanked by SDs and subtelomeric regions, and 3) quantitative PCR-based assays focused on chromosome 7. In conclusion, we have evaluated a large number of patients with AD with a 3 step approach that includes clinical characterization, cytogenetic and molecular studies and new screening assays in search of unknown genome-wide rearrangements.

Joubert Syndrome(JS) and Meckel-Gruber syndrome(MGS) are the same disorder in the Hutterite Brethren.

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JS is an autosomal recessive disorder characterized by hypotonia, ataxia, mental retardation (MR) and a molar tooth sign (MTS) on MRI. JS related disorders (JSRDs) combine features of JS plus variable ocular, renal, hepatic and limb involvement. Three loci for JS/JSRDS have been mapped but no genes have been identified. A small number of patients with a JSRD have been reported with homozygous deletions of the gene NPHP1 associated with juvenile nephronophthisis. MGS is characterized by encephalocele, renal cysts and polydactyly. Three loci for MGS have been mapped, but no genes have been identified. MGS has been reported in the Hutterites, a genetic isolate living on the Canadian prairies. Most cases are lethal, making clinical/genetic studies difficult.

We have seen 7 Hutterite patients who have a characteristic dysmorphic face, severe MR, oculomotor apraxia and a MTS (or cerebellar vermis hypoplasia on CT) suggestive of a JSRD. Three of these patients had previously been diagnosed with MGS on the basis of encephalocele and enlarged cystic kidneys (2 patients) or Dandy-Walker malformation and cystic kidneys in one sibling. The remaining four patients have facial and oculomotor features, MR and MTS, consistent with a diagnosis of JS. All 7 patients are closely related and have the same facial phenotype with MTS. We believe they have the same disorder. In this population a mutation in a single gene likely causes the JSRD/MGS phenotype, which highlights the overlap between these conditions, and suggests a common genetic pathway. Genetic modifiers may be responsible for the variability. Using homozygosity mapping, we expect to identify a novel gene responsible for this phenotype. Thus far we have excluded the 3 known JS loci and the three MGS loci. We have also excluded a homozygous NPHP1 deletion.

Shwachman Diamond Syndrome: A behavioral phenotype? *E.N. Kerr¹, A. Dupuis¹, E. Krane¹, L. Ellis¹, S. Hoosen-Shakeel¹, S. Newton^{1,2}, J.M. Rommens^{1,2}, P.R. Durie^{1,2}.* 1) Hospital for Sick Children, Toronto, ON; 2) University of Toronto, ON.

Shwachman Diamond Syndrome (SDS) is a rare recessive disease caused by disruptions to 7p12-q11 (SBDS gene). RNA analyses revealed that SBDS is expressed in all organs. Published data on cognition in SDS are limited by small numbers and poorly defined phenotype and were completed before identification of the gene. **AIM:** To determine if a behavioral phenotype is evident in SDS. Neuropsychological testing was completed with 31 children (ages 6.3-16.9 yrs) who met the diagnostic criteria for SDS (exocrine pancreatic and bone marrow dysfunction) and whose genotype was defined. **RESULTS:** Cognitive functioning of children with SDS was significantly lower than normative samples ($p < .01$) across most domains. 51.6% performed Below Average (< 25th percentile) in intellectual functioning; 19.4% performed below the 2nd percentile while only 9.7% performed Above Average (> 75th percentile). One or no child performed Above Average on higher order language, speed of mental flexibility, sustained and dual attention, and visual-motor integration. Cluster analyses revealed 5 primary classifications ranging from low to high overall performance and differentiated children with the two most common mutations 258+2T>C/183_184TA>CT (n=14) and those with 183_184TA>CT +258+2T>C/258+2T>C (n= 3) from those with 258+2T>C/rare mutation (n=9) and 258+2T>C/ 258+2T>C (n=1). Subjects with 258+2T>C/unknown mutation (n=4) were represented in each cluster. Controlling for family environment, paired analyses between unaffected siblings (n=12) and children with SDS revealed significant deficits in reasoning, problem solving, mental control, dual attention, language, academics, and visual-motor integration. Parents reported that children with SDS display significantly more problems with cognition, hyperactivity, internalizing and externalizing behaviors as well as significantly lower adaptive living skills (e.g., motor, social /communication, community and broad independence) than siblings. Although intra-group variability in performance was evident, the results do suggest probable cognitive consequences of SBDS dysfunction.

H63D homozygous mutation in a patient with hereditary hemochromatosis and Parkinson's disease. R.G.

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Hereditary hemochromatosis (HH) is a genetic disease of iron deposition in various organs of the body, including the brain. Classic medical teaching states that HH does not impact the brain symptomatically; however, several groups have anecdotally reported patients with HH and Parkinson's disease (PD) or parkinsonism; additionally, MRI findings suggest excess iron deposition in the basal ganglia in patients with HH and PD. However, very little risk information is available regarding particular genotypes and Parkinsonian phenotypes. In the limited reports where genotype data are available, the genotype for these patients was homozygous for the Cys282Tyr (C282Y) variant. We present a case of a patient who is homozygous for His63Asp (H63D) and has HH and PD, the first known report for this association.

Möbius sequence : clinical and pathogenic analysis of 68 patients. *M.J. Marques-Dias¹, J.A. Paz¹, D.R. Bertola², L.M.J. Albano², M.B. Moreira², C.H. Gonzalez³, E. Kuczyński⁴, M. Valente², C.A. Kim².* 1) Neurology, University of Sao Paulo Medical School, Sao Paulo, SP, Brazil; 2) Genetics and Radiology Units, Dept. of Pediatrics, Inst. da Crianca, Sao Paulo, SP, Brazil; 3) Department of Biology, Institute of Biosciences, University of So Paulo, So Paulo, Brazil; 4) Psychiatry Institute, Hospital das Clinicas, Sao Paulo, SP, Brazil.

Möbius sequence (MS) is a rare entity related to genetic or environmental etiologies, acting between 4-6 post conception weeks. A vascular disruption in the territory of the subclavian artery and/or its ramifications has been proposed as the main pathogenic mechanism in cases of ambient etiologies such as drug exposition and maternal traumatism. In Brazil a large number of MS patients have been recently reported in relation with misoprostol misuse in the first trimester of pregnancy. The authors present the main abnormalities in 68 patients with MS evaluated between 1991 and 2004. The 68 patients (36 female/32 male) have been submitted to clinical examination in a multidisciplinary basis in order to know the main etiologic factors, the complete clinical spectrum of abnormalities and the possible pathogenic factors. MS has been diagnosed in the presence of uni or bilateral VI and VII cranial nerve palsies. Forty-four children (65%) have been exposed to misoprostol in utero, and in 5 children a traumatic maternal event in the 1st trimester has been referred; 75% of the mothers presented bleeding at the same period (30 in the group with misoprostol and 21 without misoprostol). Besides the VI/VII nerve palsies in all patients, the XII nerve was affected in 44%. In 53 patients MS was associated with extremity defects and in 19 with hydrocephaly. Autism was diagnosed in 12.5% of the patients. Brain stem calcification was detected by CT in 28% and in 3 other cases through neuropathology study. The authors believe that MS associated to extremity defects and hydrocephaly is due to a vascular disruption between 4-6th post conception caused by misoprostol or by other traumatic event. Autistic behavior recently reported in other series of MS should add evidence to the potential structural and functional sequelae of early brain stem lesions.

Apolipoprotein E 4 is associated with risk of dementia in Inclusion Body Myopathy-Paget Bone Disease-Frontotemporal Dementia syndrome. *S.G. Mehta¹, S. Xiong², G.D.J. Watts¹, G. Umberger², M. Lovell², C.D. Smith², V.E. Kimonis¹.* 1) Division of Genetics, Children's Hospital, Boston, Boston, MA; 2) Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY.

Valosin Containing Protein (VCP) is an AAA (ATPase Associated with a variety of Activities) protein. Recently, mutations in the VCP gene were identified as the cause of a rare complex syndrome combining limb-girdle/inclusion body myopathy, Paget disease of bone and frontotemporal dementia (IBMPFD; Watts et al, 2004). We have demonstrated that VCP mutants affect the ubiquitin proteasome degradation pathways in the pathogenesis of the inclusion bodies seen in muscle and bone disease. Since IBMPFD is only 30% penetrant for the frontotemporal dementia (FTD) phenotype at average age 53 years (Watts et al 2004), additional genetic factors may be involved in FTD pathogenesis or age of clinical expression when associated with VCP mutations. We chose APOE as a putative modifier gene, as it is known to be a risk factor for Alzheimers disease, diffuse Lewy body disease and vascular dementia. APOE is not a proven risk factor for sporadic FTD, however. Clinical, biochemical and mutation analysis was carried out on 174 individuals including 61 affected, 28 carriers and 85 control patients followed by genotyping for APOE. Several of the patients have had detailed neuropsychological evaluations or neuropathological examinations to document the presence or absence of FTD. Comparisons were made between affected individuals with FTD, affected without FTD and normal controls. There was a significant difference in frequency of APO 4 alleles in affected patients with FTD ($p=0.0001$). Seven patients with FTD and IBMPFD had at least 1 APOE 4 allele ($p=0.05$). Homozygosity for APO 4 allele was further associated with FTD (3/5, 60%, $p=0.001$). One autopsied individual with VCP mutation, clinical FTD phenotype, and APO 4 allele had many neuritic plaques identified in the brain. These data suggest APOE 4 genotype may modify FTD pathogenesis or age of onset when associated with VCP mutations. This may provide clues to the pathogenesis of sporadic FTD and be helpful for counseling.

Intracranial Anomalies detected by Imaging Studies in 37 Cases of Apert Syndrome. *J.B. Mulliken*^{1,3}, *F. Quintero-Rivera*^{2,3}, *C. Robson*^{1,3}, *V. Kimonis*^{1,3}. 1) Children's Hospital, Boston, MA; 2) Massachusetts General Hospital Center for Human Genetic Research, Boston, MA; 3) Harvard Medical School, Boston, MA.

This study was undertaken to determine the frequency of agenesis of the corpus callosum (ACC) in Apert syndrome. It was prompted by the prenatal detection of ACC and hypertelorism at 19 weeks gestation in a male who was found to have Apert syndrome associated with the common S252W (934C>G) FGFR2 mutation. We report brain imaging in 37 cases of Apert syndrome evaluated at Children's Hospital, Boston. The sample included 27 males and 10 females with an age range of 2 months to 42 years. The findings reported in 14 MRI and 31 CT (8 patients had both) studies in these 37 patients included ACC in 2 (5%), hydrocephalus in 6 (16%), ventriculomegaly in 28 (76%), absence of the septum pellucidum in 7 (19%), and encephalocele in 4 (11%). Chiari I malformation was detected in 7/14 (50%) cases who had an MRI. Less common features included gyral abnormalities and undermyelination of white matter in 2 patients respectively (5%), and heterotopia of the gray matter, and interhemispheric cyst in 1 patient. Complete agenesis of the corpus was found in another male who was diagnosed at birth with Apert syndrome (S252W mutation) after a prenatal history of ACC and ventriculomegaly. In conclusion, a wide range of neuroimaging abnormalities are present in Apert syndrome suggesting that the FGFR2 gene is important in the development of the brain. Also in view of the association of ACC seen in 5% cases, we suggest that prenatal finding of ACC should lead to consideration of Apert syndrome as a possible diagnosis. A careful search by 3D ultrasound imaging for symphalangism and the associated craniofacial findings including brachycephaly, exorbitism and open metopic sutures may aid in accurate prenatal diagnosis.

Abnormal brain structure in children with nonsyndromic clefts of the lip and/or palate (NSCLP). *P.C. Nopoulos, J. Murray, J. Canady, L. Richman, L. Lehn.* Psychiatry, University of Iowa Carver College of Medicine, Iowa City, IA.

In a previous study on brain structure in adult males with nonsyndromic clefts of the lip and/palate (NSCLP), subjects with clefts were found to have abnormally enlarged anterior regions of the cerebrum, smaller than normal posterior regions of the cerebrum, and smaller than normal cerebellum volumes. To better understand the early manifestations of these abnormalities, this study is a preliminary report on the brain structure of CHILDREN (ages 7-12) with NSCLP using Magnetic Resonance Imaging (MRI).

Subjects: To date, 40 children with NSCLP have been collected and are compared to 40 healthy control children. Brain scans are processed by sophisticated software to obtain automated measures of the cerebrum, its four lobes, and the volume of the cerebellum. ANCOVA is used, controlling for the effects of age and gender.

Results: Like the previous sample of adults, children with NSCLP have significantly altered brain structure compared to controls. However, the pattern in childhood has both similarities and differences to the pattern seen in adults. For example, like adults, children with NSCLP have abnormally small volume of the cerebellum. However, unlike the adults who had larger than normal volumes of the frontal lobe, children in this study had significantly smaller than normal volumes of the frontal lobes. In contrast, posterior regions of the cerebrum were significantly larger than healthy controls.

Discussion: Children with NSCLP have significant alterations in the structure of their brain compared with controls. With the exception of smaller than normal cerebellum volumes, the pattern of cerebral brain structure is strikingly different compared to adults with NSCLP. This dichotomy between the brain structure of children with NSCLP and that of adults with NSCLP, suggests an abnormality of normal brain development, in particular late maturational processes that occur from childhood through the second decade of life.

Holoprosencephaly studies in France : mutation review and genotype-phenotype correlation. *S. Odent*¹, *C. Dubourg*², *L. Pasquier*¹, *L. Lazaro*¹, *M. Blayau*², *M.R. Durou*², *C. Bendavid*², *V. David*². 1) Genetique Medicale, Hopital SUD, Rennes, France; 2) Genetique Moleculaire, Hopital Pontchaillou, Rennes, France.

Holoprosencephaly (HPE ; 1/16.000 live births ; 1/250 conceptuses) is a common development defect affecting both the forebrain and the face. Clinical expressivity is variable, ranging from a single cerebral ventricle and cyclopia to clinically unaffected obligated carriers in familial HPE. The disease is genetically heterogeneous but additional environmental agents also contribute to the etiology of HPE. In our cohort of 250 non chromosomal HPE families, the probands were classified in 3 groups : typical HPE (49 per cent with 54 fetuses and 68 children), atypical forms or HPE spectrum (25 per cent), and polymalformative HPE (26 per cent). We studied SHH, ZIC2, SIX3, TGIF genes by D-HPLC and direct sequencing and found 38 heterozygous mutations and 1 deletion (16 per cent of all probands and 24 per cent of typical HPE), 27 of them being novel ones : 20 in SHH, 8 in ZIC2, 8 in SIX3 and 3 in TGIF. There were 17 familial cases and 22 sporadic cases; a mutation was identified in 23 per cent of familial cases. The most original and various phenotypes were associated with a mutation in SHH: hypoplasia in the pituitary gland, colobomatous microphthalmia, cerebellar hypoplasia, choanal or nasal pyriform aperture stenosis, solitary medial maxillary incisor. With a ZIC2 mutation, face was normal; one case was associated with a syntelencephaly, 2 cases with a neural tube defect. With a SIX3 mutation, ophtalmologic findings were frequent; the first case with atelencephaly was described and no other associated malformations were found. With TGIF, a cleft lip was present in the 3 families, in parents and children. This study confirms the extremely variable phenotypes in HPE families and the genetic heterogeneity of the disease. We are just starting to perceive a certain degree of genotype-phenotype correlation.

First occurrence of Aprosencephaly/Atelencephaly and Holoprosencephaly in a family with a SIX3 gene mutation. *L. PASQUIER*^{1,3}, *C. DUBOURG*³, *M. GONZALES*⁴, *L. LAZARO*¹, *V. DAVID*³, *S. ODENT*¹, *F. ENCHARAZAVI*². 1) Clinical Genetics, Rennes University Hospital, France; 2) Service d'Histo-Embryologie et Cytogénétique, Necker University Hospital, Paris, France; 3) Laboratoire de Génétique Moléculaire, Rennes University Hospital, France; 4) Laboratoire de Foetopathologie, Saint-Antoine University Hospital, Paris, France.

Aprosencephaly/Atelencephaly (AP/AT) refers to a spectrum of rare severe forebrain malformations, characterized by absent or rudimentary prosencephalon or its derivatives, leading to extreme microcephaly. The question whether AP/AT is the consequence of a destructive process, or due as Holoprosencephaly (HPE) to an abnormal patterning of the neural tube is still open. However, the occurrence of AP/AT and HPE in the same family nor a common causing gene have never been documented. This is the first report on the occurrence of AP/AT and HPE in a family with three affected sibs carrying a maternally inherited mutation of SIX3. In our panel of 210 unrelated HPE patients, mutational analysis led to the identification of seven SIX3 mutations (representing 3.3%). Phenotype/genotype correlation in this series of SIX3 mutations found a preferential association of HPE with eyes abnormalities, but no other case with AP/AT. Our neuropathological and mutational analysis with genotype phenotype evaluation bring strong evidences on the link between AP/AT and HPE and the role of SIX3 gene in the genesis of AP/AT. Furthermore, we conclude on the essential role of SIX3 in the patterning of the anterior fate of neuroectoderm and in craniofacial and visual field development. In term of timing, AP/AT may be considered as an early defect of posterior-to-anterior patterning of neural tube, affecting the differentiation of the prosencephalon into diencephalon and telencephalon and their derivatives. Further studies and specially the timing and pattern of SIX3 gene expression during early human development should help to understand the mechanism of AP/AT and associated malformations.

Megalencephalic Leukodystrophy with Subcortical Cysts: A report of two siblings and a review of the literature.

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Megalencephalic leukodystrophy with subcortical cysts (MLC) is a rare autosomal recessive disorder characterized by macrocephaly, slowly progressive spasticity and ataxia, an unusually late onset of cognitive decline, and distinctive white matter changes on MRI. We present two siblings, ages 15 and 12 years, in whom a pair of novel mutations in the MLC1 gene were identified and review 120 previously reported cases in the literature. The 15 year old sister initially presented at age 5 months with macrocephaly and normal development except for a lag in head control. A CT scan identified lucency of the white matter. By age 3 years her FOC was 54 cm. At age 12 she had a generalized tonic-clonic seizure. She was doing well in a regular classes. Her exam showed mild hypertonicity in the lower extremities and unsustained clonus. At age 15, she was strikingly macrocephalic (FOC 64 cm). Her exam was normal except for mild bilateral clonus. She had been held back one year in school and was presently in regular classes with some tutoring. Her brother first presented at age 6 months with macrocephaly (FOC 49 cm) and normal development. He had two seizures at age 37 months and a third several months after minimal head trauma. Hyperactivity and oppositional behavior were reported. After being seizure free and off anticonvulsant medication for several years, he had a recurrence at age 8 after trivial head trauma. By age 9, his FOC was 61.5 cm, and he had difficulty walking. His exam showed spasticity, hyperreflexia, clonus and poor tandem gait. He required special education classes. Their MRIs at ages 15 and 12, respectively, were nearly identical and showed decreased T1 signal and increased T2 signal throughout the supratentorial white matter with sparing of the corpus callosum and geniculocalcarine tracts. Cysts were present in both anterior temporal lobes. Sequencing of the MLC1 gene showed one allele to have a R84H mutation resulting in substitution of a histidine for arginine at position 84 and the other allele to have a canonical splice site mutation following exon 5 (538 + 1ga).

Further delineation of the clinical phenotype associated with mutations in *OPHNI* gene: a clinically recognizable syndrome. *N. Philip*¹, *B. Chabrol*¹, *C. Raybaud*¹, *C. Moraine*², *M.F. Croquette*³, *V. Layet*⁴, *A. Moncla*¹, *L. Villard*⁵. 1) Depts de Génétique Médicale, Neuropédiatrie et Neuroimagerie, Hôpital d'Enfants de la Timone, Marseille, France; 2) Hôpital Bretonneau, Tours, France; 3) Hôpital Saint-Antoine, Lille, France; 4) Hôpital Charles Nicolle, Rouen, France; 5) Inserm U491, Faculté de Médecine Timone, Marseille, France.

A mutation in the *OPHNI* gene, encoding Oligophrenin, has been reported for the first time in a family of non-specific XLMR. Since then, it has been demonstrated that mutations in *OPHNI* were associated with syndromic mental retardation with cerebellar hypoplasia in two families and a sporadic case (Philip et al, 2003; Bergmann et al, 2003). These findings were confirmed by the reappraisal of the first family, demonstrating similar MRI findings. We report here a new family with the same phenotype. We made a review of the literature in order to propose a better definition of the clinical phenotype. Neuroradiological findings were identical in all male patients: cerebellar hypoplasia predominating on the lower vermis, cortical atrophy and variable ventricular dilatation requiring ventriculo-peritoneal shunting in two cases. Mental retardation was constant, usually moderate (IQ around 50), predominating on language. In all cases tested, verbal IQ was significantly lower than performance IQ, and all had rather conserved visuo-spatial abilities. There was no associated ataxia, except in one family. Strabismus was present in three families, epilepsy in two. In addition, we demonstrated that all male patients exhibit a characteristic facial appearance. Heterozygous females exhibit mild mental retardation and slight facial dysmorphism. All five mutations are non-sens mutations or small frameshift deletions. No recurrent mutation or hot-spot was observed, demonstrating that a complete mutation screening of the gene is mandatory in suspected cases.

Cerebellar anomalies: review of a series of 83 children. *S. ROMANO*¹, *N. BODDAERT*², *I. DESGUERRE*¹, *F. BRUNELLE*², *A. MUNNICH*¹, *P. de LONLAY*¹. 1) Department of Medical Genetics, Hopital Necker-Enfants Malades, Paris; 2) Department of Radiology, Hopital Necker-Enfants Malades, Paris.

We report extensive clinical, radiological and biochemical investigation in a series of 83 children with congenital cerebellar abnormally MRI evidence of. All patients but five had isolated cerebellar abnormalities. A classification of patients was made based on the clinical and radiological aspects of the cerebellum in order to obtain homogeneous groups despite the well known variability of cerebellar findings. Five groups were identified: i) cerebellar hypoplasia without cerebellar atrophy ii) cerebellar atrophy without hypoplasia iii) cerebellar atrophy combined to hypoplasia, iv) isolated cerebellar dysplasia v) partial vermian agenesis associated (group A) with a posterior fossa cyst or not (group B). In the group B, 10 children with cerebellar vermian agenesis consistent with Jouberts syndrome presented with partial agenesis of median and inferior vermis and superior vermian dysplasia. Retinopathy was present in all patients while no renal anomalies were associated. Two sisters have an Oro-Facio-Digital syndrome (type VI) with sus-tentorial anomalies including bilateral ventricular dilatation. Apart from these two sisters, 7 patients were born to consanguineous parents. Loci of Joubert syndrome in chromosomes 9q34.1 (CORS 1), 11p12-q13.3 (CORS 2) and 6q23 (COR 3) are now tested in these inbred families.

Variability of expression of neurofibromatosis 1 in monozygotic twins. *E.K. Schorry, B.T. Tinkle.* Cincinnati Childrens Hosp, Cincinnati, OH.

Twin studies have historically been a valuable tool in studying genetic traits and disorders. We present a study of monozygotic (MZ) twins with neurofibromatosis 1 (NF1) in an attempt to better understand which of the variable complications of NF1 are genetic in origin (either due to NF1 mutation or modifying genes) and which are due to non-genetic events. We reviewed medical records and examined 6 sets of MZ twins followed at our NF clinic. All twins met diagnostic criteria for NF1; zygosity was determined by testing of 9 polymorphic markers from blood samples. Features evaluated included: presence or absence of each of the 7 diagnostic criteria of NF1; osseous complications; learning disabilities (LD) or mental retardation (MR); severity score; and MRI findings. The twins ranged in age from 1 to 15 years at time of last examination. We found a high degree of concordance in skin manifestations, including numbers of cafe-au-lait spots. Two sets of twins were concordant for specific LD, and one pair concordant for mild MR. Orthopedic complications were surprisingly concordant in the twins: 2 pairs were concordant for pectus excavatum and 2 pairs concordant for scoliosis. MRI findings of T2 hyperintensities were concordant in 2/3 pairs of twins tested. Specific tumors were generally discordant among twins. One set of twins was discordant for optic nerve glioma, and two pairs were discordant for plexiform neurofibroma. Data from twins was also compared to data from same-gender siblings with NF1. In reviewing our data and comparing to other twin sets published in the literature, we conclude that NF1 complications such as cutaneous manifestations, LD, and orthopedic abnormalities were highly likely to be concordant, implying potential importance of modifying genes. Tumors, including optic nerve gliomas and plexiform neurofibromas, which likely require a second hit mutational event, were generally discordant among the twins, implying that random factors play a role in these tumors. Study of larger numbers of twins may be valuable in further elucidating these factors.

Overgrowth, developmental delay and refractory seizures- a variant of Sotos syndrome? P. Shah¹, D. Chitayat². 1) Dept Pediatrics and; 2) The Prenatal Diagnosis Program, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada.

We saw a 3.5 years old first child of healthy, non-consanguineous parents aged 34 (height 160 cm) and 35 years (height 160 cm) at the time of birth. Pregnancy was following an ICSI procedure. There is family history of open spina bifida (maternal side), hydrocephalus in paternal sister and developmental delay and seizures in a child of another paternal sister. There was no history of alcohol, cigarette smoking or illicit drug exposure. After an elective C/S, infant required minimal resuscitation and cried at 30 sec. Apgar scores were 6 and 10. The BW was 3.75 kg (50th-75th cent), other parameters are not available. Infant was found to be unusually quiet during initial period. Excessive weight gain was noticed from 2-3 month onwards (exclusively breast fed) with all recording 97th centile. Currently, his weight is 28.7 kg, length is 111 cm and skull circumference is 53.5 cm (all 97th centile). He is currently bottle fed and does not receive any solids. Physical examination revealed high forehead, frontal bossing, bushy eyebrows, long eyelashes, depressed nasal bridge, anteverted nostrils, left undescended testis, three caf au lait spots, bilateral club feet, pes cavus, hourglass toe nails and tapering phalanges. He had first seizure at the age of 3 months following DPT immunization. He has multiple recurrent seizures requiring multiple antiepileptics. He is severely delayed (functioning at 2-3 month level) in all aspect of development. He startles to noise and does not make eye contact. He has normal metabolic screen, bone age, chromosomes, echocardiogram and Hb electrophoresis. He had negative screen for genetic markers of fragile X, SNRNP, Prader Willi, CGD, NSD1, Soto syndrome, ATR-X gene. He is on thyroxine supplement for hypothyroidism. MRI revealed delayed myelination, generalized cerebral atrophy, moderately dilated ventricles, thin and stretched corpus callosum. In summary, we present a case similar to the reported case series of variant of Sotos syndrome (1) with an additional feature being refractory seizures. 1. Amiel J. J Med Genet 2002;39:148-52.

Screening for HPE associated deletions in a child with pseudotrisonomy 13. A. Shanske¹, D. Cordero¹, C. Bendavid^{2,3}, B.R. Haddad³, M. Muencke². 1) Ctr for Craniofacial Disorders, Children's Hosp. at Montefiore, Albert Einstein College of Medicine, Bronx, NY; 2) Medical Genetics Branch, National Human Genome Research Institute, NIH, Bethesda, MD; 3) Inst. for Molecular and Human Genetics/Lombardi Comprehensive Cancer Center, and Depts. of Oncology and Obstetrics and Gynecology, Georgetown University Medical Ctr., Washington, DC.

Holoprosencephaly (HPE) is the most common structural anomaly of the forebrain. The underlying etiology is extremely heterogeneous with known teratogens and genetic factors such as visible cytogenetic anomalies, including trisomy 13, in 25-50% of newborns and gene mutations in 20-25% of chromosomally normal infants. Cytogenetic deletions and translocations have helped define HPE minimal critical regions and identify several HPE genes. Cohen and Gorlin (1991) proposed the term pseudotrisonomy 13 syndrome to designate cases that clinically resemble trisomy 13 syndrome but had normal chromosomes. There have been 26 cases reported in the literature with several cases where consanguinity suggests autosomal recessive inheritance. Genetic heterogeneity including an undetected microdeletion or dominant new mutations have been suggested. We utilized a panel of 6 BAC FISH probes to search for microdeletions in a child with pseudotrisonomy 13. JA was the 3655 gm product of a term pregnancy born to unrelated parents. A brain anomaly was identified prenatally. Postnatally, an MRI revealed semilobar HPE with multiple large extra-axial interhemispheric loculated cysts. A VP shunt was placed at 3 months because of increasing head size. His physical examination at 14 months revealed a dysmorphic severely developmentally delayed youngster with macrocephaly, bilateral epicanthal folds, hypoplastic ala nasae, and a prominent capillary hemangioma over the forehead. Polysyndactyly (pre- and post-axial) involved all 4 extremities. Laboratory investigation included a normal male GTG-banded karyotype and cholesterol. Our multicolor FISH probe panel (containing BAC probes for 6 HPE candidate genes: SHH, TGIF, ZIC2, SIX3, DISPATCHED, HNF3, Zic2) revealed no microdeletions. This study represents the first attempt to screen a patient with pseudotrisonomy 13 for a deletion of an HPE candidate gene.

A newly described type of lissencephaly in an extended consanguineous Israeli Arab family. *R. Shenhav¹, M. Mahajna², G. Halpern¹, S. Shalev³, M. Shohat^{1,4}.* 1) Medical Genetics, Rabin Medical Center Beilinson Campus, Petah Tikva, Israel; 2) Pediatric Neurology, Schneider Children's Medical Center of Israel, Petah Tikva, Israel; 3) Genetics Unit, Ha'emek Medical Center, Afula, Israel; 4) Sackler Faculty of Medicine, Tel Aviv University, Israel.

We report 2 related consanguineous Israeli Arab families who have together 5 boys with severe microcephaly. This appears to represent a new type of lissencephaly that is transmitted by autosomal recessive inheritance. All 4 parents are healthy. In one family the parents are second cousins - they have one healthy daughter as well as 2 affected children. In the second family the parents are first cousins. They have 3 affected sons, 2 of them twins, but no healthy children. The wife in the first family and husband in the second family are siblings, and the husband in the first family and the wife in the second family are second cousins. In all the affected boys the clinical picture and the course of the illness were similar. One child was still alive aged 11 months, but all the other affected children died between 2.5 and 8 months of age. Ultrasound examination in the third trimester revealed microcephaly, IUGR and limb abnormalities. Birth weight was <5th percentile. Clinical characteristics included severe microcephaly (-4.5SD), dysmorphic facial features with a low sloping forehead, micrognathia, prominent nasal bridge, prominent helices, bilateral simian crease, flexion contractures of both hands with overriding fingers, partial syndactyly of the fingers and dorsiflexion of both feet with overriding of the toes. Other features were arthrogryposis, rigidity of the lower limbs, convulsions, choanal atresia and bilateral polar congenital cataracts. MRI of the brain showed microcephaly, a small brain, pachygyria and a thin corpus callosum. The younger boy of the first family had a normal karyotype, 46,XY, and FISH showed no deletions in 17p13.3. Histology revealed lissencephaly, with a thick cortex, reduced white matter, and abnormal lamination with 4 layers instead of 6. Cerebellar histology was normal. Linkage studies are being undertaken.

Association of HOXD1 and GBX2 allelic variants with autism spectrum disorders. *C.J Stodgell¹, M. O'Bara¹, S.L. Hyman², S. Bryson³, P.M. Rodier¹*. 1) OB/GYN, Univ. of Rochester, Rochester, NY; 2) Pediatrics, Univ. of Rochester, Rochester, NY; 3) Pediatrics, IWK Health Centre, Dalhousie Univ., Halifax, Nova Scotia, Canada.

Autism spectrum disorders (ASDs) are developmental disorders with an estimated prevalence of 3-6/1,000. Genome scans have suggested linkage to Chr. 2q. We tested two candidates located on Chr. 2q for their association with ASDs in. HOXD1 (2q31.1) is important in the formation of the abducens nerve, and has been linked to Duane syndrome, a congenital abnormality of eye muscle innervation that has been reported in autism was genotyped in. We identified two SNPs in noncoding regions of HOXD1 of 188 unrelated cases of ASD and 144 unrelated controls. The first SNP is a G>C SNP 391 bases upstream of the start codon and the second SNP (T>C) is located at base 125 of the intron; both met allele frequency expectations and there was no significant difference between the cases and controls. Of 322 cases and controls where genotypes for both SNPs were available the G allele of the 3' SNP and the T allele of the intronic SNP were linked 94% of the time. GBX2 (2q37), is also a transcription factor important in maintenance of the isthmic organizer (the mid/hindbrain division) and formation cerebellar vermis. Analysis of GBX2 upstream of the start codon revealed 2 alleles that differed by 12 bases (ACAGACGGCGGG). The frequency of the short allele was .75 in 206 unrelated cases and .54 in 200 controls. Comparisons of the short allele between cases and control indicated that significant difference with the short allele being more common in cases than in controls, OR=5.09 (95% CI, 3.58-8.01); p=.00009. We also calculated that the short allele was in transmission disequilibrium (Z=5.02). These data further support the hypothesis that genes responsible for brainstem and cerebellar formation are important candidates for the etiology of ASDs. (Supported by grants to PMR 1PO1HD35466, a Collaborative Program for Excellence in Autism, and 1RO1HD34969.).

Subset of Individuals with Autism and Macrocephaly Associated with Germline PTEN Tumour Suppressor Gene Mutations. *Z. Talebizadeh¹, M.G. Butler¹, M.J. Dasouki¹, X.P. Zhou², M. Brown¹, T.N. Takahashi³, J.H. Miles³, C.H. Wang⁴, R. Stratton⁵, R. Pilarski², C. Eng^{2,6}.* 1) Dept Genetics, Children's Mercy Hosp, Kansas City, MO; 2) Clinical Cancer Genetics Program, Human Cancer Genetics Program, Comprehensive Cancer Center, Division of Human Genetics, Department of Internal Medicine, The Ohio State University, Columbus, OH; 3) Department of Pediatrics, University of Missouri, Columbia, MO; 4) Department of Neurology, Stanford University Medical Center, Stanford, CA; 5) Southwest Genetics, San Antonio, TX; 6) Cancer Research UK Human Cancer Genetics Research Group, University of Cambridge, UK.

Autism is an early onset neurodevelopmental disorder defined by significant impairment in communication and social interaction accompanied by a pattern of repetitive or stereotypical behaviors and interests. Macrocephaly is also seen in 20% of autistic patients. Autism is genetically and phenotypically heterogeneous and several genome-wide linkage analyses have shown the involvement of at least 15 genes from different chromosomes including chromosome 10. However, the genetic etiology of autism remains elusive. Individuals with Cowden syndrome and other related disorders are characterized by germline PTEN mutations and occasionally have neurobehavioral features resembling autism and also macrocephaly. The PTEN gene is localized to chromosome 10q23. We hypothesize an association between macrocephaly and autism and PTEN gene mutations. Therefore, we undertook PTEN gene mutation analysis in 18 subjects with autism spectrum disorder and macrocephaly. Of these 18 autistic subjects (13 males and 5 females; ages 3.1 to 18.4 years) with a head circumference range from +2.5 to +8.0 SD, 3 males (17%) carried germline PTEN mutations. These 3 probands had previously undescribed PTEN mutations, H93R (exon 4), D252G (exon 7) and F241S (exon 7). These three residues were highly evolutionarily conserved. We suggest that PTEN gene testing be performed on patients with autistic behavior and macrocephaly. The gene findings may impact on recurrence risks as well as medical management for the patient.

Principal signs and symptoms of NF1 patients attended at the CNNF Brazil. *G. Carakushansky^{1, 2}, M. Geller^{1, 2}, K. Higino^{1, 2}, D.R. Azulay¹, G. Coutinho², M. Coutinho², J.G. Rangel Goncalves¹, A. Bonalumi Filho², M. Suchmacher², E. Kahn¹.* 1) Dept Pediatrics, Federal Univ Rio de Janeiro, Rio de Janeiro, Brazil; 2) Teresopolis Medical School, Rio de Janeiro, Brazil.

Introduction: Neurofibromatosis type 1 (NF1) or von Recklinghausen disease is an autosomal dominant disorder caused by mutations in the 17q11.2 gene. It presents complete penetrance and a large variation in the expression of the occurrence of manifestations both intra and interfamilyally, as well as in an individual at different stages in life, as it is age-dependent. Diagnosis is based on the establishment of clinical criteria. Men and women are equally affected.

Material and Methods: Descriptive, observational, transversal study consulting the Centro Nacional de Neurofibromatose (CNNF) database, located at Instituto de Dermatologia da Santa Casa de Misericórdia in Rio de Janeiro and at the Instituto de Pediatria e Puericultura Martago Gesteira of the Federal University of Rio de Janeiro, from 1998 to 2004.

Results: 166 NF1 patients were attended, 93 females and 73 males, and plexiform neurofibromas were observed in 33%; subcutaneous neurofibromas in 56%; cutaneous neurofibromas in 60%; café-au-lait spots between 05 and 15mm in 80%; café-au-lait spots larger than 15mm in 74.69%; axillary freckling and other folding regions in 40%; Lisch nodules in 15%; and sensorineural auditive loss in 7%.

Conclusions: The majority of patients are female, predominance of cutaneous neurofibromas and café-au-lait spots between 05 and 15mm, a large proportion present freckling, Lisch nodules and sensorineural auditive loss are present in a minority of NF1 patients in Brazil.

Prevalence of Plexiform Neurofibromas at the CNNF Brazil. *M.G. Ribeiro¹, M. Geller^{1, 2}, K.S. Higino^{1, 2}, G.P. Coutinho², M.P. Coutinho², L.G.J. Darrigo², G. Carakushansky^{1, 2}.* 1) Genetics Department, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; 2) Teresopolis Medical School, Rio de Janeiro, Brazil.

Introduction: Neurofibromatosis 1 (NF1) is an autosomal dominant disorder caused by mutations to the 17q11.2 gene. The NF1 gene is a tumor suppressor, and is related to the appearance of specific lesions, such as cutaneous, subcutaneous, or plexiform neurofibromas, freckling, optic gliomas, Lisch nodules, and bone dysplasias. The plexiform neurofibroma constitutes one of the significant complications of NF1 that may occur in childhood, rarely developing after adolescence. Studies relate a prevalence of 15%, however there are divergences, with other studies relating a prevalence of 16 to 40% in NF1 patients.

Materials and Methods: Descriptive, observational, transversal study consulting the Centro Nacional de Neurofibromatose (CNNF) database, located at Hospital Geral da Santa Casa de Misericórdia do Rio de Janeiro and at the and at the Instituto de Pediatria e Puericultura Martagão Gesteira of the Federal University of Rio de Janeiro from 1998 to 2004.

Results: 166 NF1 patients were attended, 93 female and 73, male and 33% (55 cases) of plexiform neurofibromas were observed. The distribution of the plexiform neurofibromas by sex was 60% (33 cases) for females and 40% (22 cases) for males; while the age distribution was 7.27% (4) from 0 to 5 years of age; 16.36 (9) from 6 to 12; 18.8% (10) from 13 to 21 years; 47.27% (26) from 22 to 49 years, and 10.91(6) in patients older than 50.

Conclusion: In Brazilian patients, plexiform neurofibromas are predominant in female patients above 20 years of age.

Hemizyosity at the NCF1 gene in Williams-Beuren syndrome patients decreases their risk of hypertension. A. Antonell, M. Del Campo, L.F. Magano, F.J. Munoz, R. Flores, M. Bayes, L.A. Perez Jurado. Genetics and Cell signaling Units, Dept of Experimental Sciences, Universitat Pompeu Fabra, Barcelona, Spain.

Williams-Beuren syndrome is a neurodevelopmental disorder with multisystemic manifestations due to a segmental aneusomy at chromosomal band 7q11.23. Hemizyosity at the elastin gene is known to be the cause of the vascular stenoses and thought to predispose to the hypertension present in about 50% of patients. The most common ~1,55 Mb deletion is mediated by non-allelic homologous recombination between large blocks of segmental duplications. These blocks contain functional genes and pseudogenes, specifically several copies of two genes (NCF1 and GTF2IRD2), the number of which varies depending on the location of the deletion breakpoints. Fine mapping of these deletions analyzing several paralogous sequence variants was performed in order to investigate potential associations with some of the phenotypic differences present in these patients. The sample included 84 sporadic patients with an identical size deletion at 7q11.23. Significant associations were found among clinical features and molecular deletion subtypes. Particularly, high blood pressure, which was not correlated with the severity of cardiovascular disease, was more common in patients with no deletion of the NCF1 gene ($p < 0.005$). NCF1 encodes a cytosolic subunit of the leukocyte NADPH oxidase (p47phox), an enzymatic complex that catalyzes the production of superoxide (O_2^-) from NADPH and oxygen. To gain insight in the molecular mechanism by which NCF1 gene dosage correlates with blood pressure, we compared the activation of the respiratory burst (nitroblue tetrazolium test) by phorbol myristate acetate treatment (1M) in Williams-Beuren syndrome cell lines with and without NCF1 gene deletion. As expected, patients hemizygous at NCF1 gene showed less activity of the NADPH oxidase activity and thus less production of superoxide ($p < 0.005$). However, it is still unknown whether or not the decreased production of superoxide contributes to increased vascular nitric oxide bioactivity. Therefore, our findings highly suggest that deletion of NCF1 protects against high blood pressure in patients of Williams-Beuren syndrome.

Neurofibromatosis-Noonan syndrome: molecular evidence of the concurrence of both disorders in a patient. D.

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Noonan syndrome (NS) is an autosomal dominant disorder characterized by short stature, dysmorphic facial features, webbed neck, sternal deformity, heart defect, cryptorchidism in males and bleeding diathesis. The gene responsible for NS (PTPN11) was recently identified. Neurofibromatosis type 1 (NF1) is another autosomal dominant disorder characterized by café-au-lait spots, neurofibromas, Lisch nodules, axillary and inguinal freckling. The NF1 gene encodes a GAP-related protein, which acts as a negative regulator of the Ras-mediated signal transduction pathway. A clinical overlap between both syndromes named neurofibromatosis-Noonan syndrome (NFNS) is unequivocal. Four possible explanations for this combination were proposed: chance concurrence of NS and NF1; NFNS is just an unusual variant of the NS; NFNS is a somewhat unusual type of NF1; NFNS is a new entity. So far, a molecular study of the NF1 and PTPN11 genes in NFNS patients showed mutations only in the NF1 gene. We studied a 14-year-old patient with typical findings of NF1 and NS for mutations in the PTPN11 and NF1 genes. Her father was considered clinically affected by NS, with mild features. Mutational analysis for the PTPN11 gene showed an arginine to glutamine substitution at position 510 of the protein (R510Q) in the proband and her father. In the NF1 gene, a missense mutation in exon 16 was identified changing a leucine for an arginine at position 844 of the protein (L844R). This mutation was not present in her parents. The patient here reported inherited a PTPN11 gene mutation from the father and also has a de novo mutation in the NF1 gene, showing, for the first time, molecular evidence for the concurrence of both disorders in an individual, suggesting genetic heterogeneity in NFNS.

Genotype-phenotype correlation in Marfan syndrome. *A.H. Child, J. Aragon-Martin, P. Comeglio.* Cardiological Sciences, St George's Hospital Medical School, London, United Kingdom.

Purpose : To look for genotype-phenotype correlation in Marfan syndrome spectrum of disorders (fibrillin-1 deficiency).

Method: Over 600 mutations are reported in fibrillin-1 gene, 123 discovered in our clinic population. Screening for 347 consecutive patients was carried out using SSCA and /or dHPLC. Twenty-two patients were analysed using both techniques. Abnormal fragments were sequenced. One hundred chromosomes from control individuals established whether mutations were polymorphisms.

Results: One hundred and twenty three FBN-1 mutations were identified, 10 of which recurred, 32 had already been described and 91 were novel. One hundred and thirty three relatives of 60 probands were tested for carrier status, including 5 newborns (4 proven unaffected). Classic Marfan syndrome cases yielded a detection rate of 52/72 (72.2%) while a lower yield was observed from marfanoid phenotype (58/206 or 28.2%). No mutations were detected in 36 patients with isolated aortic aneurysm. In predominant ectopia lentis the mutation yield was 13/33 (39.4%).

Conclusion: FBN-1 mutation detection in the Marfan syndrome spectrum aids diagnosis, prognosis and management. Mutations in exons 24-32 are linked with neonatal Marfan syndrome, mutations at calcium-binding sites usually cause major disruption in fibrillin-1 function as do cysteine substitutions. In ectopia lentis, mutations are most often found in the first 15 exons and do not involve cysteine substitutions.

The genetic epidemiology of familial arrhythmogenic right ventricular cardiomyopathy/ dilated cardiomyopathy in Newfoundland. *G.G. Cook¹, K.A. Hodgkinson¹, S. Connors², L. Thierfelder³, A.S. Bassett⁴, P.S. Parfrey¹.* 1) Patient Research Centre, Memorial University, St. John's, Newfoundland, Canada; 2) Division of Cardiology, Health Sciences Centre, St. John's, Newfoundland, Canada; 3) Max-Delbrück Centrum für Molekulare Medizin, Berlin, Germany; 4) Clinical Genetics Research Program, Centre for Addiction and Mental Health, University of Toronto, Ontario, Canada.

We have previously shown that Newfoundland families with arrhythmogenic right ventricular cardiomyopathy (ARVC) linked to a founder haplotype at 3p25 (ARVD5) have a significantly increased mortality in males, with an overlap between an ARVC and dilated cardiomyopathy (DCM) phenotype. To determine its contribution to familial ARVC/DCM we studied 46 families referred to the Newfoundland provincial genetics clinic with ARVC, DCM or sudden cardiac death (SCD) under 50 years who had at least 2 family members with manifestations of cardiomyopathy. Of these, 25 families had at least 3 affected members in 2 successive generations and at least 2 in one sibship. 17 (68%) families were linked to ARVD5 and 8 (32%) families had ARVC/DCM/SCD not linked to 3p25. Age to death, or last follow up, was determined in family members with an a priori 50% risk of inheriting ARVC/DCM. Mortality was significantly greater in male subjects from ARVD5 families compared to families not linked to 3p25: Median age to death was 52y (95% CI 47-57; n=266) vs. 65y (95% CI 58-72; n=90): relative risk (RR) 2.2 (95% CI 1.4-3.4), p=0.0004. Results were not significantly different for female subjects: median age to death 75y (95% CI 69-81; n=206 ARVD5) vs. 90y (95% CI 67-113; n=77 not linked to 3p25): RR 1.4 (95% CI 0.7-2.7). Irrespective of linkage to 3p25, in densely affected ARVC/DCM/SCD families, males at 50% risk die significantly earlier than females (p=0.0405; non-3p25 linked, p=0.0001; 3p25 linked). We conclude that ARVD5 is a major genetic cause of familial cardiomyopathy in Newfoundland with a significantly worse mortality in male family members of probands to that for familial cardiomyopathy unlinked to 3p25.

Assymmetric septal Hypertrophy. Report of a family case. *N. Davalos¹, V. Sanchez-Barba¹, I.P. Davalos^{2,3}, L.E. Figuera^{2,3}.* 1) Hospital Regional Dr.Valentin Gomez Farias, ISSSTE; 2) Doctorado en Genetica Humana, CUCS, U de G; 3) Division de Genetica,CIBO, IMSS. Guadalajara, Jalisco Mexico.

The first demonstration of Asymmetric Septal Hypertrophy (ASH) or Cardiomyopathy Familial Hypertrophic (HC) was described by Teare in 1958, reported the autopsy findings of sudden death in 9 young subjects. ASH in early stages produces a presystolic gallop due to an atrial heart sound, and EKG changes of ventricular hypertrophy. Ventricular tachycardia or fibrillation is thought to be the principal mechanism of sudden death in patients with hypertrophic cardiomyopathy (HC). Genetic heterogeneity for ASH has been postulated with involved genes in 20q13.3, 15q14, 14q12, 3p21.3-p14.3. CASE REPORT: The propositus 33 male years-old. Clinically he presented chest pain and dyspnea with periodicity of twice a week. Familial history of sudden death. Physical examination show: high 170 cm; weight 74 Kg; blood pressure 100/60mm Hg; at cardiac area FC 70x and an aortic systolic ejection murmur. Study of proband with the full-blown condition shows that an atrial heart sound ('presystolic gallop') and EKG changes of septal hypertrophy. The echocardiographic study revealed: 1) ASH of septum without SAM. 2) septum hypokinesia. 3) diastolic disfunction. 4) FE 75%. The purpose of this paper is to describe a familial case with ASH. This family shows at least 10 affected members in 5 generations with a sex ratio F:3; M:7. Although this case revealed an association with strenuous physical exertion or sports (Spirito 1997, Maron 2003) and more males than females were affected (Greaves 1987). The pattern of inheritance was consistent with autosomal dominant inheritance, few cases with an autosomal recessive and X-linked recessive inheritance was described. Spirito in 1997 reviewed heterogeneity of clinical and genetic features and stated that the diverse clinical and genetic features of ASH make it impossible to define precise guidelines for management (OMIM 192600). Although studies have found specific mutations associated with the various forms of this condition, genotype-phenotype studies are needed to learn more about the changes in the gene and the mode of inheritance.

In frame deletion in the fibrillin1 gene in a patient presenting a Weill-Marchesani phenotype with aortic aneurysm: extending the spectrum of fibrillinopathies? *J. De Backer¹, M. Godfrey^{1, 2}, P. Van Akker¹, P. Coucke¹, A. De Paepe¹*. 1) Medical Genetics Department, University Hospital Ghent, Belgium; 2) Department of Paediatrics, Univ of Nebraska Medical Center, Omaha, NE.

In frame deletions of more than one codon of the fibrillin1 gene (FBN1) are infrequently encountered Marfan syndrome (MFS). One 9nt and one 66nt deletion have been described in 2 patients with neonatal MFS. One 48nt deletion has been described in a patient with classical MFS. Recently, an in frame deletion of 24nt has been described in a family with autosomal dominant Weill-Marchesani syndrome (WMS). We present a 62 year old male patient with an ascending aortic aneurysm for which surgery was performed. Ten years earlier he underwent surgery on both eyes for acute glaucoma, and subsequently for cataract. Ocular examination showed very shallow anterior eye chambers. Lens dislocation was not seen. On physical examination he presented a stocky stature, a round face, stiff joints and short fingers. The patients brother and sister both died suddenly at the ages of 63 and 45 years respectively. Furthermore, both his mother and maternal uncle as well as his eldest son, suffered from bilateral glaucoma. Mutational analysis of the FBN1 gene in the patient revealed a 12nt deletion in exon 20 (c2502-2513delTGAAAGTACTTT). The patient represents an unusual, so far unique, condition combining clinical features of MFS (aortic dilatation) and WMS(ocular and skeletal features). Probably the disease segregates in the family as an autosomal dominant trait. The underlying FBN1 mutation has not been reported previously neither in MFS nor in WMS. The 12nt deletion is located in exon 20, encoding the 9th cb- EGF like domain of the FBN1 gene. It is in close vicinity to a conserved cystein residue and as such likely interferes with normal calcium binding essential for functional integrity of the FBN1 gene. This observation confirms that intragenic deletions of the FBN1 gene can cause the WMS phenotype, that the clinical definition of WMS should be expanded to include cardiovascular manifestations of MFS and nicely illustrates the continuum of clinical phenotypes associated with mutations in the FBN1 gene.

Characteristics, associations, and outcome of Absent Pulmonary Valve syndrome in the fetus. *M. Gentile*^{1,2}, *P. Volpe*², *D. Paladini*³, *M. Marasini*⁴, *A.L. Buonadonna*¹, *P. Arciprete*⁵, *F.M. Boscia*². 1) Dept Med Genet, IRCCS de Bellis, Castellana(BA); 2) Dept Obstet Gynecol, AUSLBA/04 Bari; 3) Dept Obstet Gynecol, University Federico II of Naples; 4) Dept Pediatr Cardiol, Giannina Gaslini Institute, Genova; 5) Dept Cardiac Surg, AUSLBA/04, Bari, Italy.

Objectives. To assess: A) the accuracy of prenatal diagnosis; B) the incidence of extra-cardiac and chromosomal anomalies; C) the perinatal outcome in a population of 21 fetuses diagnosed with Absent Pulmonary Valve Syndrome (APVS).

Methods. The following variables of 21 fetuses with APVS were retrieved from databases: indication for referral, gestational age at diagnosis, cardiomegaly, branch pulmonary dilatation, associated anomalies, FGR, fetal/neonatal outcome. Karyotyping was performed in 20/21 cases, with FISH analysis to detect the 22q11 microdeletion performed in 16/21 cases.

Results Prenatal diagnosis of APVS proved correct in all cases, with only 3 cases occurring not in association with TOF. Additional cardiovascular anomalies were present in 5 cases (24%). Extra-cardiac anomalies were found in 9 cases (42.8%), and were associated with chromosomal anomalies in 5. The 22q11 microdeletion was present in 4/16 cases (25%). Fetal/neonatal outcome was as follows: 9 terminations of pregnancy, 3 intrauterine deaths, 6 post-natal deaths, and 3 (14.3%) neonates alive after surgery. Cardiomegaly and marked branch pulmonary dilatation was present in 16 and 15 cases, respectively, and was associated with bronchomalacia in virtually all cases.

Conclusions. APVS can be reliably diagnosed and characterized in prenatal life. The association with major chromosomal anomalies (5/20, 25%) or 22q11 microdeletion (4/16, 25%) is consistent. The relatively poor survival rate (14.3%) is due to the high rate of terminations, associated genetic anomalies, and bronchomalacia.

Nonsyndromic familial Tetralogy of Fallot (TOF) in two families with 3- terminal frame shift mutations of JAG1.

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Tetralogy of Fallot (TOF) is the most common cyanotic conotruncal heart malformation. Genes involved and molecular pathogenesis are largely unknown. Syndromic TOF has been observed with various chromosomal disorders (trisomy 21, microdeletions 22q11.2, e.g.). Few isolated nonsyndromic sporadic or familial TOFs have been found to be associated with single gene mutations (ZFPM2/FOG2 and NKX2.5). In addition, recent observations identified the JAG1 gene mutated in Alagille syndrome (AGS) as a candidate for TOF. We report about two families of European Caucasian origin with several individuals showing TOF and being affected by truncating frame shift mutations within the 3- terminal coding region of JAG1. In family A with 3 sisters with TOF a segregating maternal frame shift mutation within exon 24 (3021-3022insGC) was detected. None of the sisters demonstrated features characteristic for AGS with exception of butterfly vertebrae and an additional rib reported for the oldest sister. The mother carrying the mutation is clinically normal and even does not show any evidence for heart malformation. In family B with 3 out of 6 sibs with TOF (1 sister, 2 brothers) and one affected son a truncating frame shift mutation within exon 26 (3599-3600insG) was detected. Also in this family none of the individuals met the diagnostic criteria for AGS. Our data indicate that even truncating mutations within the 3- coding region of JAG1 may be causally related with familial nonsyndromic TOF. Further studies in sporadic and familial cases have to reveal whether JAG1 has a significant impact on TOF. Due to the asymptomatic mother carrying the mutation and the observation of low penetrance in the large kindred with TOF and a missense mutation within exon 6 of JAG1 (Eldadah ZA et al. 2001) it would be interesting how the penetrance is being modulated by the normal JAG1 allele and/or by other genes e.g. that of the NOTCH signalling pathway.

Noonan-like phenotype and PTPN11 gene: study of 15 patients. *C. Kim¹, A.C. Pereira², J.E. Krieger², L.M.J. Albano¹, D.R. Bertola¹.* 1) Genetics Clinic Unit, Dept Pediatrics, Inst da Crianca, Sao Paulo, Sao Paulo, Brazil; 2) Laboratorio de Genetica e Cardiologia Molecular, Heart Institute (InCor), University of Sao Paulo, Sao Paulo, Brazil.

Noonan syndrome (NS) is an autosomal dominant disorder comprising short stature, facial dysmorphisms, short and/or webbed neck, cardiac defect (mainly pulmonary stenosis and hypertrophic cardiomyopathy), cryptorchidism in males and bleeding diathesis. The gene responsible for this disease (PTPN11) was recently identified and accounts for 30 to 50% of the cases diagnosed clinically as NS. A few other conditions (cardiofaciocutaneous - CFC syndrome, Leopard syndrome, Noonan-like/multiple giant cell lesion syndrome, Noonan-neurofibromatosis, Costello syndrome) share several of the clinical findings present in NS. The gene PTPN11 has been tested for as the possible responsible for these disorders and this was the case for Leopard syndrome and Noonan-like/multiple giant cell lesion syndrome. On the other hand, CFC syndrome and Costello syndrome are not caused by mutations in this gene. We studied 15 patients with a Noonan-like phenotype: 10 with CFC syndrome, 3 with Leopard syndrome and 2 with Noonan-like/multiple giant cell lesion syndrome. Mutational analysis of the PTPN11 gene was performed using DHPLC and amplicons with an aberrant elution profile were subsequently sequenced. No mutations in the coding region of the gene were found in any patients. These results reinforce that CFC syndrome is not caused by mutations in the coding region of the PTPN11 gene and the Leopard syndrome is, as NS, an heterogeneous disorder. For Noonan-like/multiple giant cell lesion syndrome, so far there is only one family studied and the mutation found in the PTPN11 (Asn308Ser) was also present in another patient with NS and apparently no history of multiple giant cell lesions, indicating that Noonan-like/multiple giant cell lesion syndrome is part of the spectrum of NS. The absence of mutations in the coding region of the PTPN11 gene in our 2 patients with Noonan-like/multiple giant cell lesion syndrome, indicates that this is also an heterogeneous disorder.

Congenital cardiac disease in 2 patients with Cranio-osteoarthropathy. *S. O'Connell*¹, *M. Suri*², *C. Hall*³, *W. Reardon*¹. 1) National Centre of Medical Gen, Our Lady's Hospital for Sick C, Dublin, Dublin, Ireland; 2) Clinical Genetics Service, City Hospital, Nottingham, UK; 3) Great Ormond Street Hospital for Children, London, UK.

Cranio-osteoarthropathy is a rare condition. Classically, signs are identifiable in infancy with clubbing of fingers, and delayed closure of the clinically enlarged cranial sutures, followed by periosteal new bone formation on x-rays of the long bones. Ultimately, joint swelling and stiffness follow, typically involving the large joints. We report two unrelated patients with features of cranio-osteoarthropathy, both of whom also had a history of congenital heart disease. Moreover, the cases reported here, manifesting significant differences in radiological features, emphasise the variability of radiological findings which can attend this condition.

Familial aplasia cutis congenita, left-sided cardiac obstructive defects, and balanced 2;19 chromosome translocation. *M.E. Pierpont*^{1,2}, *J.A. Baker*¹, *K.E. Bloom*^{2,3}, *R.R. Higgins*⁴, *A. Singh*⁵. 1) Children's Hosp, Mpls/St Paul, MN; 2) Univ Minnesota, Mpls, MN; 3) Dermatology Center For Children and Young Adults, Mpls, MN; 4) Allina Cytogenetics, Mpls, MN; 5) Children's Heart Clinic, Mpls, MN.

Aplasia cutis congenita (ACC) has been described in association with congenital cardiac abnormalities. We report two families with members affected by ACC and left-sided cardiac obstructive defects. In family 1, a 15-month-old boy has ACC and complex cardiac disease (bicuspid aortic valve, aortic stenosis, coarctation of the aorta, mitral stenosis, hypoplasia of the transverse aortic arch). His 30-month-old sister has ACC and a normal echocardiogram. An older brother died at 3 months from aortic atresia and hypoplastic left ventricle. He had no ACC. The parents have no ACC and normal echocardiograms. The maternal grandmother has mild scalp vertex alopecia. Chromosomal analyses have revealed a balanced translocation $t(2;19)(p23.3;p13.11)$ in the proband, his sister, and the mother. In family 2, an 11-year-old male proband has ACC and multiple left-sided cardiac defects (bicuspid aortic valve, coarctation of the aorta, parachute mitral valve, subaortic muscular ridge, supravalvular mitral ring). A younger male sibling died at 7 weeks from hypoplastic left heart. There is an older sister with no ACC and normal echocardiogram. The father has ACC and a normal echocardiogram. The paternal grandmother has mild ACC. The proband has a normal karyotype. These two families illustrate that investigation for the presence of variable congenital heart defects should be considered for each person with ACC. In these two families, no one has a limb defect, so confirmation of Adams Oliver syndrome (AO) is not possible. However, individuals with AO have previously been described with various forms of left-sided obstructive cardiac defects, suggesting a contiguous gene disorder. The presence of a balanced translocation in family 1 provides new information for possible localization of the gene(s) responsible for ACC, AO and left heart development at 2p23.3 or 19p13.11.

A family exhibiting arterial tortuosity syndrome displays homozygosity of microsatellite markers associated with the arterial tortuosity locus. *S.H.E. Zaidi¹, V. Peltekova⁵, S. Meyer², A. Lindinger², A.D. Paterson³, L-C. Tsui^{3, 4}, A.S. Teebi³, M.F. Ul Haque³.* 1) Dept of Medicine, Toronto General Hosp, Toronto, Ontario, M5G 2C4, Canada; 2) Universitätskliniken des Saarlandes, Kirrberger Strae, Homburg, Germany; 3) Program in Genetics and Genomic Biology, Hospital for Sick Children, Toronto, ON, M5G 1X8, Canada; 4) The University of Hong Kong, Pokfulam Road, Hong Kong; 5) Mount Sinai Hospital, Samuel Lunenfeld Research Institute, Toronto.

Arterial tortuosity syndrome is a rare autosomal recessive disorder, which is characterized by severe tortuosity and lengthening of systemic and large arteries in the affected individuals. In addition patients also exhibit hyperextensible skin and hypermobility of joints, features that are characteristics of Ehlers-Danlos syndromes. This rare syndrome has been described in several ethnically diverse families and an arterial tortuosity locus on chromosome 20q13 has recently been discovered. Here we report a family with an affected child and seven unaffected individuals. The affected girl manifests elongation and severe tortuosity of the aorta, carotid and other arteries. Additional clinical symptoms include loose skin, hypermobile joints, hernias and facial features that resemble Ehlers-Danlos syndromes. This phenotype is similar to the clinical appearance of patients, for whom the arterial tortuosity locus has been mapped. We therefore performed genotyping using the microsatellite markers that are located at the arterial tortuosity locus, for the affected child, her parents and all unaffected siblings. The affected child was homozygous for these markers, while parents and three of the five siblings were found heterozygous. Genotyping using additional markers defined the genomic interval to a 37cM region flanked by D20S885 and D20S893 microsatellite markers, within which the arterial tortuosity locus is located. No mutations were identified in the coding regions of three functional candidate genes that resided at this locus. This is the second only report of the involvement of arterial tortuosity locus in an ethnically distinct family.

Stereotypy and serotonin in children with Autistic Disorder. *R.K. Abramson*¹, *A.V. Hall*², *S.A. Ravan*², *H. Cope*³, *M.L. Cuccaro*³, *M. Pericak-Vance*³, *H.H. Wright*². 1) Dept Neuropsychiatry & Behav, Wm S Hall Psychiatric Inst, Columbia, SC; 2) Dept Neuropsychiatry, Univ. South Carolina Sch. Med., Columbia, SC; 3) Center for Human Genetics, Duke University, Durham, NC.

Stereotypy is a repetitive behavior in children with Autistic Disorder(AD). In children with AD, stereotypies may be 1)environmentally mediated (Kennedy et al,2000;Militeri et al,2003), 2)persistent and independent of environment suggesting a biologically-based etiology(Kennedy et al,2000) or 3)both environmental and biologically based. Selective serotonin reuptake inhibitors treat biologically based repetitive behaviors in AD. The purpose of this study was to evaluate whether whole blood serotonin levels (5HT) in children with AD contribute to severity of stereotypy. Diagnoses of 71 probands(average age 8.54.1yrs) from the Duke/USC molecular studies of AD, were confirmed using the Autism Diagnostic Interview-Revised. Stereotypy was measured by the Aberrant Behavior Checklist stereotypy score(ABC-S). 5HT was determined by HPLC. Children were free of medication affecting 5HT. Controlling for race[17 African Americans (AA), 54 Caucasians (C)], a trend existed towards a negative correlation between the ABC-S and 5HT, $r=-.226, df=68, p=0.060$. 5HT for AA probands($X=322.948.7SE$) was higher($t=2.215, p=0.039, df=69$) than for C probands($X=210.414.4SE$). With race appropriate means, proband 5HT was high(2SD above the mean) in 24%($n=17$) and low(2SD below the mean) in 11.3%($n=8$). In the combined subgroup, AA probands had lower ABC-S scores($X=3.5831.271SE, n=5$) than C probands($X=7.2260.679, n=20$)($F=6.388, p=0.02, df=23$). C probands with low 5HT ($X=9.6671.137$) had the highest ABC-S scores. In this small race controlled sample, a trend existed for higher 5HT and lower ABC-S scores. A larger sample is necessary to evaluate the relationship between 5HT level and severity of stereotypies in AD. In a group of children the biological contribution to the severity of stereotypy may be larger than the environmental contribution, and may point the way to targeted pharmacologic intervention. High 5HT with decreased stereotypy may point to a sub-phenotype relevant to select genes to dissect the complex etiology of AD.

Severe and Progressive Osteolysis of the Toes, Feet and Legs (Acrotarsotibial Osteolysis): A Novel Disorder or a Variant of Torg Syndrome ? *D. Aktas¹, O. Akan², M. Ozates³, N. Akarsu⁴, A. Zankl⁵, A. Superti-Furga⁵, E.*

Tuncbilek¹. 1) Department of Genetics, Hacettepe University Medical School, Ankara, Turkey; 2) Department of Radiology, Hacettepe University Medical School, Ankara, Turkey; 3) Department of Radiology, Dicle University Medical School, Diyarbakir, Turkey; 4) Department of Pediatrics Hematology, Hacettepe University Medical School, Ankara, Turkey; 5) Molecular Pediatrics, University of Lausanne, Switzerland.

We report a family with three adults sibs affected by a distinct form of osteolysis restricted to the lower limbs. The onset of disease is insidious, first changes having been noticed in early adulthood. Osteolytic changes begin at the phalangeal and metatarsal bones of the foot and not progresses proximally to involve tarsal bones, tibia and fibula. In the most severely affected of the three sibs, only a small, proximally broad and distally pointed fragment each of the tibia and fibula are left. Radiographically, the changes resemble those seen in the Torg and Winchester syndromes with but are strictly limited to the lower extremities. Notably, the hands and upper extremities are not affected. There are additional findings, such as bilateral cervical ribs and increased aeration in cranial sinuses, of doubtful significance. Skin, hair, nails and teeth were unremarkable; neuromuscular signs were not noticed. Renal function was normal. During a follow-up of five years, the osseous changes, and the resulting clinical deformation and motor disability, showed a clear progression. The parents and three sibs are clinically unaffected and no radiological abnormality on their hands was found. There is no known consanguinity and the most striking feature of this condition is its limitation to the lower extremities, although it cannot be excluded that the hands will become affected later in the course of disease. The lack of ectodermal, neuromuscular and renal changes exclude a series of syndromes associated with osteolysis. We are currently testing the hypothesis that this disorder may be allelic to the Torg/Winchester syndrome, but pending molecular results, we propose the name of acrotarsotibial osteolysis syndrome to reflect the site and progression of lesions.

Three cases with limb anomalies. *N. Almadani, F. Afroozan, Y. Shafeghati, M.H. Kariminejad.* PO Box 14665/154, Pathology & Genetics Ctr, Tehran, Iran.

Three cases with limb anomalies Two patients with Tibial aplasia-Ectrodactyly syndrome , This pattern of malformation are included : split-hand/split foot, absence of Arms&Legs. Ours patient is 18 month old male with unrelated parents that suffered from Hypoplastic /absent tibia, split hands, Hypoplastic/absent carpus, Absent metacarpus, metatarsus, phalanges & toes, Oligodactyly, bilateral patellar hypoplasia and contracted knee joints. The second case is two years old male from unknown parents that suffered from Acromelia of upper limbs, Reduction deformity of arms, Absent hand, Hypoplastic/absent carpus, Absent fingers/toes or oligodactyly, Absent metacarpus & metatarsus, Hypoplastic or absent Tibia. The second syndrome is Fibular aplasia- oligodactyly-camptomelia that reported by Hecht& Scott (1981) is included absent hands, absent foot. Our case was one years old male from unrelated parents that suffered from Reduction deformity of arm, Absent hands, Oligodactyly, absent lower limb, Bowed and hypoplastic femur, Hypoplastic/absent fibula and tibia, Absent feet& toes, Syndactyly of toes . Our two cases with tibial aplasia- Ectrodactyly are similar to patients that reported with this syndrome in references, but our case with fibular aplasia- oligodactyly- camptomelia may be similar to the femur- fibula- ulna complex (FFU syndrome) but ulna deficiency not seen in our case, And the otherhand in fibular- oligodactyly- camptomelia not seen any kind of femoral deficiency.

Umbilical cord deformation sequence in an infant with arthrogryposis multiplex congenita. *J.R. Corona-Rivera^{1,2}, M.C. Abreu-Fernández², A. Corona-Rivera¹.* 1) Laboratorio de Genética Humana, CUCS, Universidad de Guadalajara; 2) Servicio de Genética, División de Pediatría, Hospital Civil de Guadalajara Dr. Juan I. Menchaca, Guadalajara, Jal., México.

Arthrogryposis multiplex congenita (AMC), also referred to as amyoplasia, is sporadic in occurrence. The current report identifies a newborn female with AMC and umbilical cord deformational sequence (UCDS). The correlations among the clinical findings are discussed. The proposita was born to a 25 y-year-old G3, P2 mother. Threatened abortion at 4th month and decreased fetal movements were noted by the mother. The delivery was normal vaginal vertex at 40.3 weeks gestation. Birth weight was 2525 g, length was 49 cm, and OFC was 34 cm. Apgar scores were 7 and 8, at 1 and 5 minutes, respectively. Length of umbilical cord was not available. Physical examination showed symmetric contractures of the four extremities, round face, small upturned nose, and midline capillary hemangioma; shoulders were internally rotated and adducted with decreased muscle mass, elbows were fixed and extended, wrist and fingers were flexed, and also showed talipes deformity. On the left lower limb, two deep grooves were observed, as well as a mild mark of constriction on the left wrist. Radiographs showed gracile ribs and thin long bones. Karyotype and renal ultrasound were normal. Electromyography reported myopathic arthrogryposis. UCDS result from constrictive umbilical cord loops around a fetal part producing deep grooves and significant structural deformation in the underlying fetal tissues. Fetal hyperkinesia is a key theory for UCDS occurrence, since increased fetal activity is believed to determine long cords. No other causative factors related to long cords like polyhydramnios, abdominal pregnancy or fetal hypertension were noted in this case. To our knowledge, cord wrapping around a limb is occasionally seen on arthrogryposis cases, although non related to UCDS. Fetal hypokinesia-AMC associated to UCDS is an unexpected event suggesting that other unknown causative factors are needed for UCDS occurrence in this patient.

Male children with sporadic form of neurofibromatosis type-1 (NF1) are at risk for learning impairment. *F.X. Coude*^{1,2}, *C. Mignot*², *S. Lyonnet*¹, *A. Munnich*¹. 1) Departement de Genetique, Hopital Necker, Paris, France; 2) Centre de Pediatrie, Aix-en-Provence, France.

Neurofibromatosis Type-1 (NF1) is a common genetic disorder associated with a variety of medical complications, cognitive impairments, and behavioral problems.

One hundred and sixteen patients with NF1 (62 males, 54 females; mean age 11.3 years, SD 2.3) were studied in terms of learning impairment (one or more grade repetitions or school exclusions).

Seventy of 132 patients had significant learning impairment which was more frequent in male than in female patients (46 vs 24, $P=0.01$) and in sporadic than in inherited forms (47 vs 23, $P=0.01$) of the disease. In male patients with the sporadic form of NF1 the incidence of learning impairment was 83%.

The incidence of learning impairment was clearly dependent upon the mode of inheritance of NF1 and the sex of the patient. These both parameters may be taken into account in order to discriminate young children with NF1 at risk for learning problems.

A novel SOX2 mutation associated with bilateral anophthalmia. *L. de Sanctis¹, U. de Sanctis², L. Tornetta³, L. Razzano², F. Cresi¹, K. Williamson⁴, D.R. Fitzpatrick⁴*. 1) Dept. of Pediatrics, Torino, Italy; 2) Dept. of Clinical Physiopathology, Ophthalmic Clinic, Univ. of Torino, Italy; 3) Dept. of Infant Neuropsychiatry, Regina Margherita Children Hospital, Torino, Italy; 4) MRC Human Genetics Unit, Edinburgh EH4 2XU, UK.

SOX2 is a transcription factor with site- and stage-specific expression in the developing eye and nervous system. Four SOX2 mutations have been described in subjects with bilateral anophthalmia and anophthalmia/microphthalmia, the rarest and most severe structural eye defect. SOX2 mutations account for 10-15% of these disorders and other genetic and environmental factors have been implicated. EYY was born at 41 weeks of gestation from unrelated healthy parents after an uneventful pregnancy, weighing 3260g and with a head circumference of 34 cm. At birth he was found to have bilateral anophthalmia with small palpebral fissures, confirmed at 3 months by MRI scan, which also showed rudimentary optic nerves and hippocampal and parahippocampal malformation. No significant craniofacial dysmorphism were present, apart from a prominent antehelix bilaterally. At 16 months of age he had hyperextensible joints with axial hypotonia. His cognitive development was apparently normal. He has had one grand mal seizure associated with fever, but normal EEG. SOX2 mutation analysis, performed on genomic DNA, as previously described (Fantes et al.2003), identified a frameshift mutation c.628delA in the activation domain of SOX2. Direct sequencing both parental samples showed that this was a de novo mutation. We report the first point deletion in SOX2 gene associated with bilateral anophthalmia, thus confirming the essential role of this transcription factor in eye development. Clinical detailed study in the other SOX2 mutated subjects could clarify whether the here described craniofacial dysmorphisms, neuropsychomotor impairment and brain MRI malformations are specific.

An heterozygous mutation of the lamin A/C gene leading to a complex phenotype : acro-osteolysis, atrial fibrillation, hypertriglyceridaemia and autoimmune thyroiditis. V. Drouin¹, J. Senant², L. Maréchal^{1, 2}, P. Richard³, R. Ben Yaou³, T. Frebourg^{1,2}, G. Bonne³. 1) Department of Medical Genetics, Rouen University Hospital, 76031 Rouen, France; 2) Department of Neurology, Rouen University Hospital, 76031 Rouen, France; 3) Inserm U523, Institut de Myologie, Groupe Hospitalier Salpêtrière, Paris, France.

The *LMNA* gene encodes A and C lamins which are intermediate filament proteins of the nuclear lamina. *LMNA* mutations have been shown to be responsible for distinct autosomal dominant or recessive pathologic conditions primarily affecting striated muscle, adipocytes, or peripheral nerves or causing premature aging. Complex phenotypes have recently been described. We report in a family a new complex phenotype with dominant transmission resulting from an heterozygous *LMNA* mutation. The proband is a 50 year old women. At 35 years she developed atrial fibrillation and, at 45 years, a Hashimoto thyroiditis and hypertriglyceridaemia. Clinical examination revealed acro-osteolysis of the upper limbs, proximal muscular weakness, slight excess of subcutaneous fat in the neck and truncal region, curly hairs but her teeth and skin were normal. Radiological examination of clavicles was normal. She has two sons : one of them presented clinical isolated muscular weakness and the older one has a normal clinical examination. The proband's brother has distal acro-osteolysis, atrial fibrillation and hypertriglyceridaemia and the proband's mother, deceased, had distal acro-osteolysis, atrial fibrillation and conduction defect. Molecular analysis of the *LMNA* gene revealed an heterozygous mutation (R335W) in the proband and her two sons. Acro-osteolysis is one of the skeletal abnormalities described in mandibulo-acral dysplasia, an autosomal recessive disorder caused by homozygous mutations of the *LMNA* gene, associated in some cases with partial lipodystrophy. This feature has not been described so far in patients with *LMNA* heterozygous mutations. This case emphasizes the importance of complete clinical and biological evaluation in patients with laminopathy.

Hypercalciuria and nephrocalcinosis in infants with Osteogenesis Imperfecta. *E.R. Elias¹, P. Zeitler²*. 1) Dept Genetics & Pediatrics, Children's Hosp, Denver, CO; 2) Department of Endocrinology Children's Hosp, Denver, CO.

Excessive urinary calcium excretion and nephrocalcinosis have been reported in patients with OI. However, little is known about how the bisphosphonate pamidronate, now widely used to treat OI patients, may effect urinary calcium excretion in this patient population. We examined nephrocalcinosis and urinary calcium excretion in a population of infants with OI treated with pamidronate.

Methods: Nine young children with OI were studied between 1/01 and 6/04. The patients included 2 females with very severe OI (biochemically Type II/III), 5 female patients with severe phenotype (biochemically Type III or III/IV), and 2 patients (one female, one male) with mild phenotype (Type I). The patients received IV pamidronate every 2 months, starting at age 2 months, at a dose of 0.5 mg/kg/d for three days. A random urinary Ca/Cr ratio was determined. Renal ultrasounds were obtained at baseline and every 6 months. Serum calcium and renal function were also assessed.

Results: All patients with severe or very severe phenotype developed hypercalciuria by 6 months of age, defined as Ca/Cr 0.2. The Ca/Cr ranged from 0.75-1.44 by one year of age. After one year, with continued pamidronate therapy, the Ca/Cr ratio fell in all patients, and reached normal range in three patients by age 2 years. Both patients with Type II/III OI developed nephrocalcinosis by age 1 year, as did one phenotypically severe patient with Type III OI. The nephrocalcinosis resolved by age 2 years in two patients, and improved in the third. The two patients with mild OI had normal Ca/Cr and normal renal ultrasounds. All patients maintained normal serum calcium and renal function.

Conclusions: Hypercalciuria was present in all seven patients with severe OI phenotypes and nephrocalcinosis developed in three of these patients. The elevated calcium excretion and renal calcium improved over time with pamidronate use. Further study is warranted to determine if these findings can be replicated in a larger number of patients, and if this apparent effect of pamidronate is dose or age dependent.

Two brothers with findings resembling Pseudo-TORCH syndrome: Further widening of the phenotype. C.

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Intracranial calcification and microcephaly are frequently associated conditions with a heterogeneous pathogenesis. Two brothers of non-consanguineous parents are reported who presented with microcephaly at 20 weeks gestation. Post-delivery both brothers developed hyperbilirubinemia, disseminated intravascular coagulation, renal failure and died in the neonatal period. At post mortem there was periventricular calcification. Skeletal survey and microscopy of the psoas muscle was normal. Congenital infection screen, chromosome analysis and interferon-alpha levels in the CSF were normal. Blood lactate was raised but there was no evidence of systemic cytochrome oxidase deficiency on testing. Prenatal presentation of microcephaly with hyperbilirubinemia and thrombocytopenia and normal CSF interferon-alpha, may make Aicardi- Goutieres (AGS) less likely. Hoyeraal-Hreidarsson syndrome is associated with thrombocytopenia and microcephaly. Mutations in the DKC1 gene have been identified in this condition. Mutation analysis was performed in the second brother, but was negative. The brothers would fulfil the diagnostic criteria for pseudo-TORCH syndrome, but only two previously described families have had children who died either shortly after birth or in utero. Only approximately half of the AGS families show linkage to chromosome 3 and the recent description of three AGS-like patients with normal interferon-alpha and high CSF neopterin and biopterin suggests genetic and phenotypic heterogeneity. AGS and pseudo-TORCH may be allelic and identification of genes associated with these conditions will allow the elucidation of the underlying molecular and biochemical mechanisms in these diseases.

Neurofibromatosis 1 or isolated Lisch nodules? Case report. *M. Geller*^{1, 3}, *K.S. Higino*^{1, 3}, *S. Kac*², *G. Carakushansky*^{1, 3}, *M.G. Ribeiro*³. 1) Teresopolis Medical School, Rio de Janeiro, Brazil; 2) Hospital dos Servidores do Estado do Rio de Janeiro, Brazil; 3) Federal University of Rio de Janeiro, Brazil.

Introduction: Neurofibromatosis 1 (NF1) is an autosomal dominant condition caused by mutations on the NF1 (17q11.2) gene, presenting complete penetrance and a wide variety of manifestations. Diagnosis is based on two or more NIH criteria: six or more café-au-lait spots; two or more neurofibromas of any kind or plexiform neurofibroma; axillary or inguinal freckling; optic glioma; two or more Lisch nodules; distinct bone lesion (long bone pseudoarthrosis or sphenoid wing dysplasia); first degree relation with NF.

Lisch nodules represent the most frequent ophthalmologic alteration in NF1 patients. In the presence of other NF1 diagnostic criteria or a positive family history, their presence confirms NF1 diagnosis. In the absence of other signs, their presence is highly suggestive of NF1. Lisch nodules are extremely rare in the general population, and as such they are practically a pathognomonic sign of NF1.

Case Report: RSG, female, age 14 years 9 months, first pregnancy of young, non-consanguineous parents, no family history of NF1. Was referred to the CNNFUF RJ due to three café-au-lait spots larger than 15mm, two smaller than 05mm, numerous bilateral Lisch nodules, marfanoid phenotype, ocular hypertelorism, psychomotor agitation alternated with periods of tranquility, and learning disability. Born with congenital crooked foot, surgically corrected at 9 months. Presented neuropsychomotor development delays (walked at 14 months, talked at 18 months), menarche at 12 years, convulsive febrile crises in early childhood. Currently in high school, presents karyotype 46, XX and negative innate metabolism errors.

Conclusion: Strong suspicion of NF1, although not meeting the NIH criteria. We highlight that this may be an atypical or late onset case of NF1, in which many of the classic manifestations of NF1 are not present until the end of the third decade of life; requires clinical follow-up.

Parry Romberg Syndrome. Two report cases in different mexican families. *C.N. Gil^{1,4}, J.M.R. Aparicio^{2,4}, W.B. San Martin³, N.C. Hernandez¹, E.M. Huitzil⁴, F.C. Salinas⁵*. 1) Estomatology; 2) Genetics; 3) Maxilar Surgery, Hospital Para el Nino Poblano Puebla, Mexico; 4) Estomatology Benemerita Universidad Autonoma de Puebla; 5) Oral Genetics Medical University, South Carolina, USA.

Introduction. Parry-Romberg Syndrome is a rare disorder characterized by slowly progressive degeneration or atrophy of the soft tissues of half of the face with changes of the eyes and hair; and neurological abnormalities including episodes of seizures and severe pain in tissues supplied by the fifth cranial nerve including the mouth, cheek, nose, and/or other facial tissues (trigeminal neuralgia). In individuals with the disorder, initial facial changes usually the tissues above the upper maxilla and progress to involve the angle of the mouth, the areas around the eye, the brow and the ear. In addition, the skin overlying affected areas may become hyperpigmented or with vitiligo. Many individuals also experience atrophy of half of the upper lip and tongue as well as abnormal exposure. Clinical cases. Two male patients were evaluated at the department of genetics, a 9 and 16 years old males with the clinical manifestations mentioned above. Apparently, there is not medical background in their families and both are de novo genetic affected. In most cases, appears to occur randomly for unknown reasons (sporadically). However, several familial cases have been reported, which suggest that the disorder may be inherited as an autosomal dominant trait. In some cases, prior trauma in the affected area may play some role in predisposing certain individuals to the disorder. moreover, Parry-Romberg Syndrome appears to affect more females than males. There is no cure and there are no treatments that can stop the progression of Parry-Romberg syndrome. Reconstructive or microvascular surgery may be needed to repair wasted tissue. The timing of surgical intervention is generally agreed to be the best following exhaustion of the disease course and completion of facial growth. Most surgeons will recommend a waiting period of one or two years before proceeding with reconstruction. Muscle or bone grafts may also be helpful. Other treatment is symptomatic and supportive.

Clinical characterization of esophageal atresia with/without tracheoesophageal fistula in newborn infants. H.

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Esophageal atresia with/without tracheoesophageal fistula (EA/TEF) is a life-threatening malformation that occurs in 1/3,000 newborns. This condition occurs as an isolated anomaly, or occurs frequently, with other malformations and as a part of recognized syndromes. The molecular and genetic pathogenesis of EA/TEF remains unknown. In clinical setting, infants with EA/TEF are still associated with significant morbidity and mortality. In order to describe the clinical picture of newborn infants with EA/TEF, we reviewed the findings of 37 patients with EA/TEF that were treated at Nagano Children's Hospital (NCH) over a 10-year period. Polyhydramnios recognized in 68% of cases. The mean birth weight was 2227 g (range, 1017-3360 g). Gross Type C was most common (76%). Eleven newborns (30%) were isolated cases; 26 (70%) had associated malformations. Among 26 infants with associated anomalies, 14 were diagnosed: Trisomy 18 (8), VACTERL association (3), other chromosomal abnormalities (2), and hemifacial microsomia (1). Cardiac (67%), tracheo-broncho-pulmonary (33%), skeletal (33%), and gastrointestinal anomalies (17%) were identified in undiagnosed patients with associated anomalies. Besides of Type H patients, gastrostomy was performed in all patients and 30 patients had subsequently end-to-end primary repair. All isolated cases survived, but survival rate was 46% in patients with associated anomalies. Among those, Trisomy 18 accounted for 70%. Late complications were found in 80% of survival patients. Those included postoperative leakage, stenosis, gastroesophageal reflux, and feeding difficulties. Family history was negative in all cases. The prognosis and mortality largely depend on presence of other associated anomalies and systemic syndrome. A precise diagnosis is crucial for the prognostic information and management. Dysmorphological evaluation by clinical geneticists for other associated malformations including VACTERLs features together with appropriate chromosomal analysis is essential.

Exclusion of Fras1 gene mutation in patients with MOTA Syndrome and report of 8 new cases in Manitoba aboriginal and a Dutch Patient. C. Li^{1,4}, A. Slavotinek², J. van de Kamp³, S. Marles¹, C. Greenberg¹, B. Chodirker¹, A. Chudley¹. 1) Sect Genetics & Metabolism, Univ Manitoba, Winnipeg, MB, Canada; 2) Div Clinical Genetics, Univ California, San Francisco; 3) Dept Clinical Genetics, Leiden University, The Netherlands; 4) Genetics Program, University Health Network, Univ Toronto.

The Manitoba Oculotrichoanal (MOTA) Syndrome is characterized by the presence of an aberrant anterior wedge-shaped hairline, unilateral upper eyelid coloboma or cryptophthalmos, hyper-telorism, broad or notched nasal tip and anal anomalies. The condition is particularly prevalent in the Aboriginal of Island Lake region of Northern Manitoba. Since the first report of 6 children [Marles et al., 1992], 7 more patients of Cree/Ojibway background have been identified. Ocular, anal and hair anomalies are not always observed. Omphalocele, a feature previously not identified, is present in 3 of them. Furthermore, two of the patients have bilateral, instead of unilateral, abnormal anterior hairline and eye anomalies. Development is essentially normal in these patients. A Dutch girl was recently identified as probably having this condition. She has bilateral coloboma of the upper eyelids, absent upper fornix of the left eye, broad nasal tip with palpable groove. All patients examined to date have normal karyotypes. The fact that these patients have hypertelorism, grooved nasal tips, anal anomalies and omphalocele suggests a midline developmental defect. There is some clinical overlap, but major differences to Fraser syndrome, an autosomal recessive condition with classical features of cryptophthalmos, syndactyly, ambiguous genitalia, laryngeal and genito-urinary malformations, and mental retardation. Mutations in FRAS1 gene have been demonstrated to cause Fraser syndrome. We screened two patients with MOTA syndrome for mutations in the FRAS1 gene by sequencing and no mutations were identified. Recently, a large Turkish kindred with individuals having features consistent with MOTA syndrome were identified. Linkage analysis using this family excluded the involvement of FRAS1 gene in these patients. (personal comm, A. Tukun, Ankara Univ, Turkey).

The Role of a Genetics Evaluation in the Patient with Craniosynostosis. *M.J. Lyons, K.M. Chen, A. Kwan, L. Hudgins.* Pediatrics/Medical Genetics, Stanford University, Stanford, CA.

Craniosynostosis is common with an incidence of 1 in 2000 births. The cause is unknown in the majority of cases with approximately 10-20% attributed to genetic syndromes. The purpose of our study is to determine the utility of a genetics evaluation for patients referred with a diagnosis of craniosynostosis. Patients referred over a 7 year span who had possible craniosynostosis were assessed. The results for 62 patients were included. A total of 23 individuals were referred with a presumptive diagnosis of a craniosynostosis syndrome. A genetics evaluation led to a change in the diagnosis in 43% of these patients, including 50% of cases referred with a diagnosis of Crouzon syndrome (n=10), Pfeiffer syndrome (n=4), and Saethre-Chotzen syndrome (n=2). Even in Apert syndrome, the diagnosis was changed based on a genetics evaluation in 2 of 7 patients. Overall, a genetics evaluation led to a definitive diagnosis for 33 (53.2%) patient referrals. The most common clinical diagnosis was Crouzon syndrome with confirmatory testing in 6 of 8 cases. A clinical diagnosis of Apert syndrome was made for 6 patients with confirmatory testing in 2 of 2 tests performed. Pfeiffer syndrome was clinically diagnosed in 3 cases with confirmatory testing in 1 of 2 tests performed. A clinical diagnosis of Saethre-Chotzen syndrome was made for 3 patients with confirmatory testing in 2 of 3 tests performed. Jackson-Weiss syndrome was suspected in 2 cases. The diagnosis was changed to Pfeiffer syndrome in one case after testing. A chromosomal abnormality was detected in 3 cases. The 8 remaining diagnoses included craniofrontonasal dysplasia (n=3), Antley-Bixler syndrome (n=2), craniodiaphyseal dysplasia (n=1), hemifacial microsomia (n=1), and Costello syndrome (n=1). Our study demonstrates a relatively high yield for a genetics evaluation with a genetic diagnosis made in over half of the referrals. The genetics evaluation allowed for the selection of appropriate diagnostic testing, leading to a more accurate diagnosis with the best use of available resources. An accurate diagnosis is critical for accurate recurrence risk counseling and prenatal diagnosis.

Cartilage-Hair Hypoplasia in the Geauga County Amish settlement of Ohio. *L. Nye¹, E. Puffenberger², M. Johnson³, A. Zinn³, H. Morton², H. Wang¹.* 1) Das Deutsch Center (DDC) Clinic for Special Needs Children, Middlefield, OH; 2) The Clinic for Special Children, Strasburg, PA; 3) Center for Human Genetics, and Departments of Genetics and Pediatrics, University Hospitals of Cleveland and Case Western Reserve University, Cleveland, OH.

Cartilage-hair hypoplasia (CHH) is an autosomal recessive form of short-limbed dwarfism caused by errors in the RMRP gene. It was first recognized as a distinct entity in the Old Order Amish, and later reported in non-Amish groups as well. However, it has not previously been found in the Geauga County Amish settlement of Ohio, the fourth largest Amish community in North America. Here we report the first case of CHH in this community and describe his unusual presentation, as well as a first report of suspected Leri-Weill dyschondrosteosis (LWD) in his maternal line. The proband was the product of a marital union between fourth cousins. Genealogic analysis showed that both maternal and paternal ancestors mostly originated from Holmes County, Ohio. The proband was a full term male with birth weight of 2840 gm and length of 39 cm. He presented with bilious emesis. Hirschsprung disease was later confirmed via laparotomy. With classical signs of CHH including skeletal features, sparse and light-colored hair, and lymphopenia, along with his ethnic origin, diagnosis of CHH was easily made. Mutational analysis of the RMRP gene established that he was homozygous for the 70AG mutation, common in the Amish. However, this family's pedigree was further complicated by maternal short stature associated with the Madelung deformity and cleft lip, and cleft lip in two of the proband's three siblings. Mutational analysis showed that the mother was heterozygous for the 70AG mutation. Her radiographic findings are suggestive of a different skeletal dysplasia, Leri-Weill dyschondrosteosis (LWD). We think it most likely that she has LWD plus an unrelated cleft lip rather than an atypical form of CHH. To our knowledge, no cases of LWD have been described in the Old Order Amish community. Further studies, including evaluation of maternal relatives and mutational analysis of the SHOX gene, which is associated with LWD, are being considered.

Novel mutations of PTPN11 in Korean patients with Noonan syndrome. *J.Y. Park¹, S.S. Kim¹, G. Kim¹, S. Park¹, J.H. Choi², I.S. Park^{1,2}, H.W. Yoo^{1,2}, Y. Kim^{1,2,3}.* 1) Genome Research Center for Birth Defects and Genetic Disease; 2) Department of Pediatrics, Asan Medical center; 3) Asan Institute for Life Sciences, University of Ulsan College of Medicine, Seoul, Korea.

Noonan syndrome (NS; OMIM 163950) is an autosomal dominant developmental disorder with incidence of 1 in 1,0002,500 live births. Clinical characteristics of NS are proportionate short stature, facial dysmorphism, skeletal anomalies, and cardiac defects. Multiple minor anomalies such as hypertelorism, downward slanting palpebral fissures, malrotated ears, webbed neck, low posterior hairline and cubitus valgus are also presented with NS. The protein-tyrosine phosphatase nonreceptor-type 11 gene (PTPN11) was as the gene mutated in this patients with NS. PTPN11 is a cytoplasmic protein with two tandemly repeated Src homology 2 (SH2) domains in the amino-terminus and a protein-tyrosine phosphatase (PTP) domain in the carboxyl-terminus. The SH2 domains are known to function as mediators for the interaction of the PTP domain with phospho-tyrosine substrates. PTPN11 is widely expressed in the most tissues and functions as a signaling molecule in regulation of variety of cellular processes such as mitogenic activation, metabolic control, transcription regulation, and cell migration. Using PCR-direct sequencing of the exonic regions of PTPN11, we identified eight heterozygous missense mutations of PTPN11 in 24 Korea NS patients, including two novel mutations, T59A and A461T, and six reported mutation, Y62D, Q79R, E139D, F285S, Y279C, and N308D. To further confirm the association of T59A and A461T mutations with NS, we tested 100 alleles of non-patients for the presence of T59A and A461T, and consequently verified that T59A and A461T are present only in the NS patients. These mutations were found in the SH2 domain or the PTP domain. These results further support that haploinsufficiency of PTPN11 is responsible for the phenotypic features of NS across different ethnics.

Congenital diaphragmatic hernia: clues to genetic causes. *B. Pober^{1,2}, A.E. Lin^{1,3}, B. Strauss¹, M. Mullen², S. Chakravorty², MN. Westgate³, J. Wilson², P.K. Donahoe¹, L.B. Holmes^{1,3}.* 1) MassGeneral Hospital for Children, Boston, MA; 2) Dept Surgery, Children's Hospital, Boston, MA; 3) Brigham and Women's Hospital, Boston, MA.

Congenital diaphragmatic hernia (CDH) is a common, often lethal birth defect. Improved treatment will likely stem from understanding the genetic causes of CDH. We analyzed all CDH cases ascertained by a single surveillance system to identify clues to genetic causation. **Methods:** Cases consisted of all infants >20 weeks gestation who delivered over a 30 year period at Brigham & Women's Hospital. Records were reviewed for classifying CDH type (Bochdalek vs. non-Bochdalek), presence or absence of associated anomalies (focusing on associated cardiovascular malformations, CVMs), and apparent etiology of CDH. Family history was also reviewed. **Results:** We identified 223 cases with CDH. Since many cases are referred to our hospital, we calculated a nonreferred CDH birth prevalence of ~2.2/10000. Among all 223 babies with CDH, 90% had a Bochdalek hernia. Half had CDH associated with other birth defects, syndromes or chromosome abnormalities, while the other half had isolated CDH. In most cases we could not identify an etiology for the CDH. Detailed analysis of CVMs showed no significant increase in any type but we classified 4/5 conotruncal defects as double outlet right ventricle. Preliminary analysis of precurrence risk showed it to be low; among 163 nonsyndromic Bochdalek hernia cases only 1/145 prior siblings had CDH. We found 9 twin pairs discordant for CDH but only one was a nonreferred case. On initial examination, we found no evidence of advanced paternal age among fathers of CDH cases. **Discussion:** These findings do not identify a specific genetic etiology for CDH. However, our low frequency of familial clustering, similar to the work of Czeizel (*AJMG*;21:1985), as well as the presence of discordantly affected twin pairs, many of whom are MZ, suggest multifactorial inheritance or de novo dominant mutations. If CVM analyses on larger cohorts confirm homogeneity among, or show an excess of, conotruncal defects then genes important for conotruncal formation might be involved in diaphragm development.

Malignant hyperthermia and genetic disorders in Brazilian families. A. Santa Rosa¹, R. Sudo², J. Cabral de Almeida³, J. Llerena³. 1) Serviço de Pediatria, Hosp. Geral de Bonsucesso, Ministério da Saúde, Rio de Janeiro, Brasil; 2) Centro de Hipertermia Maligna, Depto. Farmacologia, Universidade Federal do Rio de Janeiro; 3) Centro de Genética Médica, IFF, FIOCRUZ, Rio de Janeiro.

Malignant hyperthermia (MH) is an autosomal dominant condition affecting skeletal muscle in susceptible patients undergoing general anesthesia. Triggered by commonly used halogenate inhalatory anesthetic agents and succinylcholine, MH remains one of the major causes of anesthetic death, despite the availability of presymptomatic testing and specific pharmacologic treatment. MH susceptibility is genetically heterogeneous. The ryanodine receptor (RYR) gene, mapped on 19q13.1-13.2 (Maclennan, 1990, McCarthy, 1990) represents an important candidate gene but only about 50% of the families shows linkage to this region.

Since 1993, the Malignant Hyperthermia Center has received 121 patients from the whole country to perform the *in vitro* caffeine/halothane contracture test, the only reliable method of predicting MH susceptibility (Larach, 1989). 32 of them (26,44%) were referred due to personal history of MH crisis, 78 (64,46%) because of positive familial history, and 11 (9,4%) for having clinical/genetic conditions associated to a greater risk of developing MH, including a case of central core disease. The 66 positive patients (54,54%) were arranged in 25 families. After thorough clinical examination including complete pedigrees, the authors have found 2 families with King-Denborough syndrome, one with Noonan-like phenotype, a probable myotonic dystrophy patient, mother of two affected siblings, and a patient with Kabuki make-up syndrome, a previously undescribed association.

Knowledge of MH susceptibility status before undergoing surgery is of utmost importance. That is why we consider that clinical geneticists working together with pharmacologists can surely be of great help in diagnosing previously unnoticed genetic conditions in families with known pharmacogenetic diseases like MH, reporting new associations, and selecting within those families the best subjects for further mutation studies on the RYR1 gene and others involved on the MH phenotype.

Pacman dysplasia is a prenatal form of mucopolipidosis type II (I-cell disease). *R. Saul¹, V. Proud², H. Taylor¹, J. Leroy¹, J. Spranger¹.* 1) Greenwood Genetic Ctr, Greenwood, SC; 2) Children's Hospital of the King's Daughters, Norfolk, VA.

Mucopolipidosis type II (ML-II) or I-cell disease is a lysosomal enzyme transport disorder in cells of mesenchymal origin. Patients with ML-II have an unremitting course, usually leading to death before ten years of age. Pacman dysplasia has been previously reported to be a lethal skeletal dysplasia with epiphyseal stippling and osteoclastic overactivity. All 4 cases of Pacman dysplasia have been recognized prior to 30 weeks gestation. We report a sibling of a fetus with Pacman dysplasia. The affected patient at 7 months of age had dysmorphic craniofacial features (prominent forehead, midface hypoplasia, depressed nasal bridge, upturned nares and slightly posteriorly rotated ears), clear corneae and scoliosis. Radiographs were consistent with dysostosis multiplex. Plasma lysosomal enzyme activities were grossly elevated for arylsulfatase-A, beta-galactosidase, and beta-glucuronidase. Fibroblast lysosomal enzyme activities were markedly reduced for alpha-mannosidase, beta-mannosidase, beta-galactosidase, and beta-glucuronidase. Lysosomal enzymes in leucocytes were normal. Intracytoplasmic inclusions were present in fibroblasts. The diagnosis of mucopolipidosis type II (I-cell disease) was firmly established. Her previous male fetus sibling had been electively terminated at 24 weeks gestation for a suspected lethal short-limbed dwarfism. Radiographs confirmed widespread stippling, femoral periosteal cloaking and osteopenia. This sibling was reported by Miller et al. (2003) as the fourth case of Pacman dysplasia. We suggest that this fetus has prenatal expression of ML-II. This wide spectrum of expression is consistent with other lysosomal storage disorders, many of which are known to cause abnormalities ranging from severe prenatal expression (lethal non-immune hydrops fetalis) to a more typical postnatal course. This prenatal form appears to expand the phenotypic spectrum of ML-II and suggests that Pacman dysplasia is not a separate entity.

A case of trisomy 13 with unusual longevity and autoimmune dysfunction. *R.E. Schnur, S. Ennis, S.N. Moore, J. Keenan.* Pediatrics/Genetics Division, Cooper University Hospital, Camden, NJ.

Long term survival beyond infancy is quite rare in trisomy 13 and there is limited data on the natural history of this disorder in long-term survivors. We describe a case of non-mosaic trisomy 13 in a 24 year-old African-American female, one of only 3 reported adult patients with this disorder. She had typical features, including unilateral cleft lip and palate, rocker bottom feet and minor cardiac defects, but no life-threatening anomalies. Menarche occurred at 13 years of age. She has had regular periods with premenstrual behavior changes, but her periods are now becoming shorter and lighter. Developmentally, she is extremely delayed. She drinks from a cup and she hand scoops her food, but is unable to use utensils. She is largely non-verbal, but says mama non-specifically and uses other sounds that seem specific to her situation. She is partially able to walk with support. She has favorite toys and responds positively to her caregivers. She has a well-controlled seizure disorder with demyelination on MRI scan. Cytogenetic analysis of two separate peripheral lymphocyte specimens (ages 3 and 17) showed a 47, XX, +13 karyotype in 20 metaphases for each screen. At age 20, she developed increased fatigue and was diagnosed and treated for hypothyroidism. Raynaud's phenomenon was noted at age 21. She has recently had difficulty maintaining her weight. She also has had numerous infections of the skin and oral mucosa, particularly with *Candida*. She now has anti-Sjögren, anti-double stranded DNA, anti-cardiolipin, and anti-Smith antibodies, and a low C4 component of complement, consistent with systemic or subacute lupus. She has also had proteinuria. Patients with trisomy 13, as with other aneuploid disorders (e.g., trisomy 21, 45,X, and 47,XXY), may be at risk for the development of autoimmune diseases.

18q Deletion Syndrome: Mimicking Non-Classical Congenital Adrenal Hyperplasia. *C.D. Trevors, D. Chitayat, M. Nezarati.* Clinical & Metabolic Genetics, The Hospital For Sick Children, Toronto, Ontario, Canada.

18q deletion syndrome consists of facial dysmorphism, mental deficiency, short stature and hypoplasia of the external genitalia in both sexes. This is a relatively rare condition with approximately 100 cases previously reported. The clinical manifestations vary according to the extent of the deletion. The condition was reported to be associated with 17,20-lyase deficiency, which results in elevated 17(OH) progesterone. A false diagnosis of non-classical congenital adrenal hyperplasia thus has been repeatedly made and treatment with corticosteroid initiated. We report a male patient, born to a 34 year-old primigravida woman of French Canadian descent and a 39 year-old father of Italian descent. The couple is healthy, non-consanguineous and their karyotypes were normal. The patient presented with a micropenis and elevated 17-hydroxyprogesterone. He was thus diagnosed as having non-classical congenital adrenal hyperplasia and was placed on cortef. No decrease in cortisol levels was noted and there were no signs of virilization or premature sexual development observed. The results of the molecular testing for congenital adrenal hyperplasia are pending. Seven cases of isolated 17,20-lyase deficiency have, however, been reported previously in children with 18q Deletion Syndrome. They had elevated 17-hydroxyprogesterone and decreased androgen levels. An isolated 17, 20-lyase deficiency should not result in a decrease in cortisol levels. There have been very few cases reported of isolated 17, 20-lyase deficiency. The validity of its existence has been questioned due to the fact that cytochrome P450c17 catalyzes both 17-hydroxylase and 17, 20-lyase activities. Mutations in the genes encoding both cytochrome P450c17 (CYP17) and 21-hydroxylase (CYP21A2) are known causes of non-classical congenital adrenal hyperplasia. Although rare, based on previous reports, the possibility of an isolated 17, 20-lyase deficiency should be explored in all individuals with 18q Deletion Syndrome prior to treatment with corticosteroids.

Adults with VATER association: long term prognosis. *P.G. Wheeler¹, D.D. Weaver²*. 1) Div. of Genetics, Nemours Children's Clinic, Orlando, FL; 2) Dept. of Medical and Molecular Genetics, IU School of Medicine, Indianapolis, IN.

VATER association is an extensively used diagnosis typically based on a constellation of congenital anomalies. Since reported long-term follow-up information on VATER association is limited, it is difficult to prognosticate the future of infants with this condition. There are some data on growth failure and mental retardation but these data are minimal [Bull et al., 1985; Mapstone et al., 1986; Weaver et al., 1986]. Since first described, the findings associated with VATER association have been expanded greatly, and now overlap syndromes such as Feingold syndrome [Feingold et al., 1997], Fanconi anemia [Perel et al., 1997], and the 22q11.2 deletion [McDonald-McGinn DM, et al., 1999]. We have undertaken a long-term follow-up of individuals reported originally by Weaver et al in 1986 or diagnosed with VATER association by his associates and him after 1986. Out of 50 potential interviewees, we were able to contact 19 individuals or families. Two of the 19 individuals had died from their birth defects: one at 3 d/o with cardiac failure due to a truncus arteriosus and one at 4 y/o, cause unknown. One was unwilling to participate. Of the rest, we interviewed and examined 7 persons, and interviewed another 9 over the phone. Of the 16, 5 had had some degree of cognitive impairment. These individuals were more likely to have congenital anomalies outside of the typical scope of VATER association, such as prune belly syndrome or features of CHARGE association. Of nine individuals with a history of imperforate anus; 5 had partial or complete incontinence as adults, which has led to difficulties in maintaining employment. Height was 5th%ile or less in 6 of 16 patients. Three of 4 patients who were trying to have children had difficulties with infertility. In 2 of the women, the infertility was thought to be related to a history of congenital anomalies of the GU system and multiple pelvic surgeries. We will further review the long-term medical and neurologic problems in these individuals, discuss the differential diagnoses associated with VATER association, and recommend a protocol for evaluating individuals suspected of having VATER association.

Non-mosaic isochromosome Yp in a male with hypogonadism and mild cognitive disability. *P.L. Wilson, S.E. Palmer, P.R. Blackett, J.Y. Lee, H. Overcash, S. Li.* University of Oklahoma HSC, Department of Pediatrics, Sections of Genetics and Endocrinology, Oklahoma City, OK.

Isochromosome Yp and Yq have been reported in a small number of patients with female, ambiguous, or abnormal male phenotypes, almost all mosaic for 45,X. We report a rare case of non-mosaic isochromosome Yp in a 16-year-old male with delayed puberty. Physical examination revealed a male phenotype with hypogonadism (small penis, mild gynecomastia, and eunuchoid appearance). The remainder of the phenotype was unremarkable with normal stature. Early development was reportedly normal. He had mild cognitive deficits and ADHD with immature behavior. Endocrine evaluation revealed a delayed bone age, normal thyroid studies, low testosterone, and low growth hormone, LH, and FSH unresponsive to GnRH stimulation. Chromosomal analysis revealed 46 chromosomes including one X and a small metacentric marker chromosome, resulting in a karyotype of 46,X,i(Y)(p10). To confirm the findings we performed FISH analysis using an SRY probe mapped to Yp11.3. Signals for the SRY gene were present on both ends of the marker chromosome, confirming isochromosome Yp. The father and three fertile paternal half-brothers were unavailable for evaluation. The young man's full brother had no reported delays in puberty or development; his karyotype was 46,XY. Certain phenotypic features of this case may reveal effects of presence, absence, or dosage of certain Y genes. One other case of i(Yp) was reported in a fetus with slight facial and foot dysmorphism. Those features are absent in this patient, and the endocrine abnormalities cannot be compared. Abnormalities of the Y chromosome in the presence of 45,X mosaicism usually results in a female or an ambiguous phenotype. The absence of mosaicism in this patient may account for his male phenotype. The dosage effect of two copies of SRY, in the absence of 45,X mosaicism, has unclear phenotypic effects. Absence of DAZ and AZF genes on Yq would predict isolated azospermia, with normal or elevated FSH. Therefore, the exact roles of the duplicated Yp and absent Yq genes of this karyotype responsible for this young man's cognitive and endocrine deficiencies are unclear.

Trigonocephaly, Polysyndactyly, Brachydactyly, Cutis Laxa and Developmental Delay: A New Autosomal Recessive Syndrome. *N. Al-Sanna'a*¹, *M. Al-Khunaizi*¹, *A.S. Teebi*². 1) Pediatrics Specialty, Dhahran Health Center, Dhahran, Saudi Arabia; 2) Division of Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto, ON.

Trigonocephaly with metopic syntosis is genetically heterogeneous. Syndromic and non-syndromic forms exist. Among the syndromic forms, an increasing number of rare autosomal recessive syndromes are being characterized together with a number of rare chromosomal syndromes. Developmental delay is relatively common. Here we report on two brothers born to a phenotypically normal first cousin parents from Saudi Arabia with another unique presumably autosomal recessive trigonocephaly syndrome. Both displayed a characteristic constellation of features that include trigonocephaly with metopic syntosis, narrowing of coronal structures, peculiar facial appearance, redundant skin with features of cutis laxa, variable limb anomalies that include polydactyly, cutaneous syndactyly and brachydactyly and mild developmental delay. Chromosomal analysis in the older brother showed a balanced translocation 46,XY,t(2;6)(12q23) while chromosomes were normal in the other. We believe that the constellation of manifestations represents a previously unrecognized autosomal recessive syndrome.

Congenital Hypertrichosis Lanuginosa. A report case in a Mexican Family. *J.M.R. Aparicio^{1,8}, C.N. Gil^{2,8}, M.P. Barrientos³, W.B. SanMartin⁴, E.R. Balbuena⁵, L.H. Hurtado⁶, M.A. Gutierrez⁶, J.L. Penaloza y Senties⁷, F.C. Salinas⁹.*
1) Genetics; 2) Estomatology; 3) Endocrinology; 4) Maxilofacial Surgery; 5) Dermatology; 6) Citogenetics and Clinical Laboratory; 7) Pediatrics Hosp para el Nino Poblano, Puebla, Mexico; 8) Dept estomatology, Benemerita Universidad Autonoma de Puebla, Mexico; 9) Dept Oral Genetics, Medical University South Carolina, USA.

Introduction. Disorders of hypertrichosis are distinguished by the distribution of hair, as well as by the temporal pattern of growth, the possible associated congenital anomalies, and the possible inheritance pattern. An X-linked syndrome of hypertrichosis associated with gingival hyperplasia has been described. Patients develop excessive body hair, but the abnormal hair is of the terminal type. In 1648, Aldrovandus first documented a family with hypertrichosis. Over the next 300 years, more than 50 reports were described, and 34 patients with congenital hypertrichosis were identified. In 1993, Baumeister et al raised the possibility that 9 of the patients with hypertrichosis had a defining clinical presentation, which he called Ambras syndrome. In 1 of the 9 patients, a specific genetic abnormality was found on chromosome 8, and the remaining 8 patients were proposed to have shared this defect. In 1998, Balducci described a patient with CHL who had a different genetic defect on chromosome 8. Clinical case. A 45 days new born patient with congenital hypertrichosis lanuginosa, was evaluated where, most of the body is covered with fine, blond or unpigmented hair at birth. There was not found abnormalities of other organ systems, although single case reports note abnormalities such as supernumerary teeth, glaucoma, aortic and cardiac valve abnormalities, and macromastia. is 1 case per 10 billion. No racial nor sex predilection exists. The pathogenesis is unknown. However, it has been refered to be inherited in an autosomal dominant manner; most cases involve a familial component. A paracentric inversion on bands 8q12 and 8q22 in one patient was reported by Balducci in 1998. No known hormonal or endocrinologic abnormalities have been identified.

The Hunter-MacDonald syndrome is associated with risk of meningioma. *L. Armstrong¹, G.E. Graham², R.N. Schimke³, D.J. Kirse⁴, F. Costello², H.H. Ardinger⁵.* 1) University of BC, Vancouver, BC, Canada; 2) University of Ottawa, Ottawa, ON, Canada; 3) University of Kansas, Kansas City, KS; 4) Wake Forest University, Winston-Salem, NC; 5) Prairie Village, KS.

The Hunter-MacDonald syndrome (HMS) has been described as an autosomal dominant pattern of congenital malformations. To date, four people in two unrelated families have been diagnosed and two other family members seem to have the condition on record review. Follow-up of these families has shown that predisposition to brain tumor is also a feature of HMS.

In HMS there is short stature, scoliosis, epiphyseal dysplasia, camptodactyly of the fingers, thumb subluxation, and malformed feet. Craniofacial features include normal head circumference, tall forehead, bitemporal narrowing, ptosis, short palpebral fissures, short philtrum, and a columella extending below the alae nasi. Decreased hearing acuity and congenital heart defects are present in most. Intelligence is normal. High resolution chromosome analysis is normal. The father of Proband 1 and the grandmother of Proband 2 died at the ages of 35 and 55 years with brain tumors. Proband 1 was diagnosed with a brain tumor at age 36 years. The asymptomatic 42 year old mother of Proband 2 was found to have a brain tumor on screening MRI. Thus, four of the six known individuals with HMS have had tumors clinically and/or pathologically diagnosed as meningiomas. The remaining two have not been screened.

Individuals with the pattern of congenital malformations of HMS should be screened for meningiomas. The diagnosis should be considered in people who develop a meningioma and have any of the typical skeletal, craniofacial, hearing, or cardiac features. Candidate genes include those associated with the multiple epiphyseal dysplasias, with signaling pathways regulating osteogenesis and tumor suppression, and with familial meningioma.

Connective tissue dysplasia in two new patients with NF1 microdeletions: further expansion of phenotype. D.

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Abstract Deletions of the entire gene have been found in approximately 5-10% patients with neurofibromatosis type 1 (NF1). The phenotype of these patients usually includes early onset and large number of neurofibromas, presence of congenital anomalies, cognitive deficiency and variable dysmorphic characteristics and growth abnormalities. Previous reports include mitral valve prolapse, joint laxity and soft skin of palms in a few patients, but connective tissue abnormalities are not generally recognized as a part of NF1 microdeletion syndrome. We describe clinical findings in two newly diagnosed patients with NF1 microdeletions (not visible by standard chromosome analysis), with presenting symptomatology of connective tissue abnormalities and typical NF1 features only appearing later. We suspect that connective tissue dysplasia is common in patients with NF1 microdeletions, and screening for associated cardiac manifestation may be warranted. Despite a large number of known patients with NF1 microdeletions, the phenotype is still incompletely defined. By reviewing available literature, we conclude that skeletal anomalies are common in this population of NF1 patients as well. More detailed clinical information and molecular studies in other individuals with large deletions involving NF1 locus may shed a light on possible genotype/phenotype correlation or may lead to identification of new genes responsible for connective tissue disorder in proximity to the NF1 gene.

The Adult Phenotype of 22q11 Deletion Syndrome (22qDS). *A.S. Bassett^{1, 2}, O. Caluseriu^{1, 4}, V. Wong^{1, 2}, M. Gatzoulis³, G. Webb^{2, 3}, R. Weksberg^{2, 4}, E.W.C. Chow^{1, 2}.* 1) Centre for Addiction and Mental Health (CAMH), Toronto, ON, Canada; 2) University of Toronto, Toronto, ON, Canada; 3) Toronto Congenital Cardiac Clinic for Adults, Toronto, ON, Canada; 4) Hospital for Sick Children, Toronto, ON, Canada.

Initial reports of clinical features in microdeletion syndromes such as 22qDS rely on paediatric data. Adults reported are most commonly transmitting parents with milder phenotypes. We systematically assessed the clinical features of 77 adults with 22qDS, ascertained through a cardiac clinic (n=32), psychiatric (n=36), genetic (n=13), or other sources (n=6) including three as transmitting parents. Subjects (36 male; 41 female), mean age 31.5 (SD 9.6) years, had 22q11.2 deletions confirmed with FISH. 59 (77.6%) were diagnosed with 22qDS as adults and variable levels of learning difficulties, dysmorphic facial features and hypernasal speech were found in most subjects. Only 33.5% had a history of palatal surgery (3.9% with overt cleft palate); 7.8% had hypoplastic thymus. Common later onset features included (% , 95% CI): hypocalcemia (55.8%, 44.5-67.2), hypothyroidism (20.8%, 11.5-30.1), thrombocytopenia (27.3%, 17.1-37.5), hearing deficit (27.3%, 17.1-37.5), severe acne (23.4%, 13.7-33), cholelithiasis (18.2%, 9.4-27), asthma (18.2%, 9.4-27), epilepsy (15.6%, 7.3-23.9), renal failure (9.1%, 2.5-15.7), and pilonidal sinus/cyst (6.5%, 0.8-12.1). The most common psychiatric disorder was schizophrenia, present in 22.6% (7-38.2) of subjects ascertained with tetralogy of Fallot. Of 22qDS subjects ascertained with schizophrenia, 33.3% (17.2-49.8) had a major CHD. Three subjects died, aged 18, 25, and 69 years; only one had a CHD. The CHD rate in adults with 22qDS is lower than previously reported when ascertainment bias is taken into account, despite using CHD as a clinical criterion for deletion testing. Understanding the adult phenotype is essential for determining rates of congenital and later onset features. More accurate frequency estimates will be important for genetic counselling and planning long term follow-up of multi-system genetic syndromes.

Three new Cases of duplication 22Q11.2 with neuropsychological problems, learning disability and subtle dysmorphic features. *S. Beiraghi¹, A. DeMarco¹, R. Lutz², K. Conway¹, K. Moller¹.* 1) University of Minnesota, Minneapolis, MN; 2) University of Nebraska Medical Center, Omaha, NE.

Duplication of 22Q11.2 region is a rare condition with a subtle physical phenotype, yet significant speech and neuropsychological findings.

Here we report on three cases with two De novo and one maternally inherited duplication of (22)(q11.2)(q11.2) with hypernasal speech, learning disabilities, Attention Deficit Hyperactivity Disorder (ADHD), visual impairments, and mild craniofacial anomalies.

Case 1 is the product of a second pregnancy to healthy nonconsanguineous parents. At age of 9 years, she was referred for concerns of hypernasal speech. Clinical examination revealed subtle dysmorphic features including hypertelorism, learning disabilities, ADHD, and significant velopharyngeal inadequacy for speech.

Case 2 is a 13 year old white female with maternally inherited dup22q11.2. Clinical examination revealed subtle dysmorphic facies, visual impairment, hypernasal speech, ADHD, and depression.

Case 3 is a 14 year old male with physical findings including hypospadias, inguinal hernia, and urthero diverticulum. His history is significant for ADHD, multiple learning and behavioral problems, hypernasal speech, and suspected schizophrenia.

Based on the subtle phenotype of these cases, 22q11.2 duplications may be rarely identified because of the lack of significant physical findings. However, given the speech disorder and neuropsychological findings associated with this genetic disorder, FISH testing is warranted for identifying these cases..

Laryngotracheal involvement and novel skeletal findings in a patient with probable Rothmund-Thomson

Syndrome: A novel RTS subset? *M.J. Borden¹, L. Armstrong², L.L. Wang³, S.E. Plon³, T. Kovest⁴, A.G.W. Hunter⁴, G.E. Graham⁴.* 1) Human Genetics Department, McGill University, Montreal, QC, Canada; 2) Children's and Women's Hospital of British Columbia and University of British Columbia, Vancouver, BC, Canada; 3) Texas Children's Hospital and Baylor College of Medicine, Houston, TX, USA; 4) Children's Hospital of Eastern Ontario and University of Ottawa, Ottawa, ON, Canada.

Rothmund-Thomson syndrome (RTS) is an autosomal recessive condition characterized by poikiloderma, hypotrichosis, juvenile cataracts, growth retardation, skeletal anomalies and increased risk of malignancy. We describe a 16-year-old French Canadian female who meets the criteria for probable RTS but has novel respiratory and skeletal anomalies. Her characteristic features are poikiloderma, scalp alopecia, generalized hypotrichosis, reduced nasal height, hypodontia, minor nail anomalies, severe feeding difficulties, early failure to thrive, short stature, small hands and feet, osteoporosis, delayed bone age and puberty, and secondary amenorrhea. She did not have the photosensitive rash seen in some RTS patients but had little sun exposure before age 4. She has no radial ray anomalies or history of cataracts, corneal dystrophy or malignancy. She has mild intellectual delay, which has (rarely) been reported in RTS. Her novel respiratory features include severe congenital subglottic stenosis, tracheo-bronchomalacia, recurrent respiratory infections and chronic lung disease. Airway malformations in RTS were first reported in a 4-year-old male with subglottic stenosis, a type I laryngeal cleft, bilateral vocal cord paralysis and recurrent respiratory infections. Skeletal anomalies are recognized in RTS, but our patient's avascular necrosis of the hip, mild leg length discrepancy, L5/S1 spondylolisthesis, anterior vertebral wedging, and hypoplastic medial malleoli are novel. Sequencing of 21 exons and 13 short introns of the *RECQL4* gene did not reveal pathogenic mutations. Southern blotting was negative for large rearrangements. Genetic heterogeneity is recognized so these patients may represent an RTS subset caused by a single gene mutation or contiguous gene deletion.

Adult psychiatric phenotype in 6p25 deletion syndrome. *O. Caluseriu*^{1, 2}, *E.W.C. Chow*^{1, 3}, *R. Weksberg*^{2, 3}, *L. Rosenberg*^{1, 3}, *D.J. MacCrimmon*^{4, 5}, *A.S. Bassett*^{1, 3}. 1) Clinical Genetics Research Program, CAMH, Toronto, ON, Canada; 2) The Hospital for Sick Children, Toronto, ON, Canada; 3) University of Toronto, Toronto, ON, Canada; 4) Hamilton Health Science Centre, Hamilton, ON, Canada; 5) McMaster University, Hamilton, ON, Canada.

Chromosome 6p deletion is a rare finding in patients with developmental delay. Anderlid et al (2003) have proposed distinct phenotypes associated with interstitial (6p22-p24 region) and terminal (6p24-pter) deletions. The terminal deletions are clinically characterized by Axenfeld anomaly, hearing loss and mid-face hypoplasia. We present an adult with clinical features of a 6p terminal deletion and a psychiatric phenotype. We assessed a 36-year-old Caucasian woman with schizophrenia and dysmorphic features. She had a long, asymmetric face, hypertelorism; low set, posteriorly rotated ears, short nose with upturned tip, high-arched palate and difficulties with coordination. The patient had a history of Axenfeld anomaly, bilateral hearing loss and mitral valve incompetence. She also presented delayed developmental milestones, mild mental retardation and articulation difficulties. She had a 7 year history of unstable mood, developed psychotic episodes with delusions and auditory hallucinations at age 33 years and was diagnosed with schizophrenia. She recently had a single generalized seizure. A CT scan showed generalized cortical brain atrophy and extensive bilateral white matter attenuation in the area of the corona radiata. There was no family history of mood disorder or schizophrenia. Diagnosis of 46, XX, del(6)(p25) was made by standard chromosomal studies (G-banding, 500 band resolution). To our knowledge, this is the first report of an adult with del 6p25 and a psychiatric phenotype. Haploinsufficiency of genes including FOXC1 in the 6p25-pter region has been proposed to contribute to the developmental delay in 6p25 deletions. Linkage studies have previously suggested the presence of susceptibility genes for schizophrenia in 6p25-p24. Patients with 6p25 deletion may be at increased risk of developing a psychiatric illness in adulthood. 6p25-pter may contain genes for susceptibility to schizophrenia.

Abdominal Visceral Findings in the Marfan Syndrome. *K. Chow, L.J. Kessler, J.E. Bavaria, H.I. Litt, R.E. Pyeritz.*
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Marfan syndrome (MFS) is a connective tissue disorder with well-characterized features in the ocular, cardiovascular, respiratory and musculoskeletal systems. Managing the aortic complications involves computed tomographic (CT) or magnetic resonance (MR) imaging. Cysts in the liver and kidneys and stones in the gall bladder were frequent and apparently incidental findings on these radiological studies. Accordingly, we reviewed CT and MR exams from an unselected cohort of patients (pts) with MFS to determine the prevalence of abdominal visceral findings and compare the findings to both published population values and exams of people being considered as renal and hepatic donors at our institution. Imaging records for 69 pts (mean age 43 yrs, range 17-75 yrs; F=28, M=41) meeting strict diagnostic criteria for MFS seen in 1998-2004 were reviewed. The presence and number of abdominal visceral findings were noted, particularly renal and hepatic masses and cholelithiasis. Dedicated abdominal CT or MR scans were available in 41 pts, while chest scans with imaging of the upper abdomen were reviewed in 28. Renal cysts were present in 41 pts (59.4%) vs 9/29 controls (31%) and 41% reported in the general population ($p=0.01$ for controls and $p=0.004$ vs population). 27.5% of pts had one cyst, while 31.9% of pts had 2 or more. The mean number of renal cysts was 4.1 in pts vs 1.0 in controls. Hepatic cysts were present in 24 pts (34.8%) vs 5/29 controls (17.2%) and 18% in the general population ($p=0.08$ vs. controls and $p=0.001$ for population). The mean number of hepatic cysts was 2.6 in pts and 0.24 in controls. Cysts were more frequent the older the pt. Cholelithiasis was present in 14 pts (20.2%) vs none in controls ($p=0.009$) and 13.8% in the general population ($p=0.116$); those with previous cholecystectomy were not included. Patients with MFS have an increased prevalence of renal and hepatic cysts, and they develop these cysts at earlier ages compared to the general population. There is a trend towards increased prevalence of gall stones. Further studies will evaluate the rate of appearance and growth in cysts and explore the pathogenesis of these visceral features.

Features of Wolfram Syndrome Without Detectable Molecular Change in WFS1. *K. Crow¹, P. Himes², L. Linck¹, R. Weleber², K. Trzupke², J. Reiss¹, R.J.H. Smith³.* 1) Dept Medical Genetics, Kaiser Permanente Northwest, Portland, OR; 2) Casey Eye Institute, Oregon Health & Sciences University, Portland, OR; 3) Molecular Otolaryngology Research Laboratories, University of Iowa Hospitals & Clinics, Iowa City, IA.

Wolfram syndrome (WS or DIDMOAD: Diabetes Insipidus & Mellitus with Optic Atrophy and Deafness) is a rare autosomal recessive condition with estimated incidence of 1/770,000. Minimum diagnostic criteria are juvenile-onset diabetes and optic atrophy by age 15 years. In addition to diabetes insipidus and high-frequency hearing loss, other features may include seizures, psychiatric illness, cognitive decline and urinary tract abnormalities. Two loci, WFS1 and WFS2, have been identified in this genetically heterogeneous condition. We report two cases with clinical diagnosis of Wolfram syndrome but without detectable pathological changes in WFS1. Case 1 is a 14-year-old girl diagnosed with diabetes mellitus at age 3, and progressive optic atrophy and bilateral high-frequency sensorineural hearing loss at 12 years. She is treated for depression and has heat intolerance due to autonomic dysfunction. Case 2 is a 28-year-old woman diagnosed with profound congenital sensorineural hearing loss in her first year, insulin-dependent diabetes at 4 years, and optic atrophy at 8 years. She developed a seizure disorder at age 12 followed by cognitive decline and mental illness. Both cases had molecular analysis of WFS1: Case 1 carried several benign single nucleotide polymorphisms (SNPs). Case 2 was heterozygous for H323R, a novel conservative amino acid change of unclear significance, and several benign SNPs. In addition, mitochondrial screening in Case 2 was negative for changes associated with MERRF, NARP, and MELAS; Friedrichs ataxia analysis established heterozygosity for this condition. While both cases meet the clinical criteria for WS, Case 1 represents a classic presentation. Case 2 exhibits unusual congenital profound hearing loss rather than typical high-frequency hearing loss diagnosed in the second decade. Interactions with other loci may contribute to the severity of hearing impairment in the second patient.

Identification of a behavioral phenotype in mouse models of Bardet-Biedl syndrome. *R.E. Davis¹, M. Fath^{1,2}, D.Y. Nishimura¹, C.C. Searby^{1,2}, M. Andrews^{1,2}, K. Wang¹, B. Yang¹, E.M. Stone^{1,2}, V.C. Sheffield^{1,2}.* 1) University of Iowa, Iowa City, IA; 2) Howard Hughes Medical Institute, Iowa City, IA.

Bardet-Biedl syndrome (BBS) is a genetically heterogeneous disorder characterized by obesity, retinopathy, polydactyly, hypogenitalism, renal abnormalities and cognitive impairment. Eight BBS genes have been identified. Our lab has developed mouse models for BBS2 (*Bbs2^{-/-}*) and BBS4 (*Bbs4^{-/-}*). Although developmental delay and learning disabilities are often stated features of BBS, there is a paucity of details regarding neurological and behavioral deficits in human BBS patients. Therefore, we carried out detailed neurological characterizations of knockout mice strains using a battery of behavioral tests (SHIRPA). Thirty-eight separate observational measurements were compared between *Bbs2* and *Bbs4* knockout, heterozygous, and wild type mice. There was no significant difference in the observations that assess muscle and lower motor neuron functions such as body position, balance and coordination, gait, positional passivity, tail elevation, grip strength, body tone or various reflexes. However, *Bbs2^{-/-}* and *Bbs4^{-/-}* mice showed deficits compared to littermate control mice in visual placement, olfaction, vocalization and touch escape. Notably, the knockout mice of both genotypes were significantly more docile during handling. The observed docility was further evaluated using a face-to-face social dominance tube test. This testing demonstrated social subordination of homozygous *Bbs2* and *Bbs4* knockout mice to heterozygote and wild type mice.

Quantification and analysis of craniofacial disorders using morphometric analysis of 3-dimension laser surface scans. *B. Dawson*^{1,2}, *S. Carter*^{1,2}, *G. Milne*¹, *A. Tran*¹, *C. Shaw*¹, *C. Bacino*¹, *B. Lee*^{1,2}. 1) Dept. of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Howard Hughes Medical Institute.

Craniofacial malformations are present in a significant portion of human birth defects. To facilitate both the speed of diagnosis and to create a quantitative and reproducible method of analyzing variation in craniofacial size and shape, we are developing Cyberware 3-dimensional laser surface scanning to gather morphometric data on affected populations with Cleidocranial Dysplasia (CCD), and control groups, and individuals with Achondroplasia. We demonstrate high reproducibility of the scanning process through using standard operating procedure.

We have developed a method to quantify the variation between CCD (N=8), Achondroplasia (N=8), and control populations (N=20), by first performing a fast 3d laser scan and subsequently marking and measuring the linear distances between a set of carefully chosen facial landmarks based on existing human craniofacial indexes. Using methodology based on 25 landmarks, over 300 unique linear distances are generated. By utilizing Euclidean Distance Matrix Analysis (EDMA), an invariant form of statistical form analysis, we generated a matrix containing every permutation of linear distance between landmarks, and measure the variance of each set of distances in comparison to each other and the norm. Data from this cohort show that statistical morphology software can differentiate between populations with and without CCD, as well as separating two separate conditions with distinct craniofacial phenotypes from each other (CCD and Achondroplasia). These data form the basis for developing new tools to quantify dysmorphology in humans.

Program Nr: 669 from the 2004 ASHG Annual Meeting

Meier-Gorlin Syndrome (Ear-Patella-Short Stature Syndrome): Growth Hormone Deficiency and Previously Unrecognized Findings. *E. Faqeih, N. Sakati, A.S. Teebi.* Department of Medical Genetics, King Faisal Specialist Hosp., Riyadh, Saudi Arabia.

Meier-Gorlin syndrome (MGS) is a rare, well-characterized autosomal recessive syndrome, associating pre and post natal growth retardation with bilateral microtia, patellar aplasia/hypoplasia, and other anomalies. Intelligence is usually normal or borderline. We report a Saudi Arabian female patient of consanguineous parents, with typical features of this syndrome in addition to respiratory difficulties including emphysematous lung changes and isolated growth hormone deficiency. Her intelligence was above average. Literature pertaining to MGS has been reviewed and the importance of growth hormone deficiency as a factor contributing to the severity of growth retardation in some cases is discussed.

Sensorineural Hearing Loss (SNHL) in a patient with Beckwith-Wiedemann Syndrome(BWS). *Y.P. Geng¹, E. Godoy¹, S. Ramanathan², L. Mehta².* 1) Queens Long Island Medical Group, Flushing, NY; 2) Schneider Childrens Hospital, Manhasset, NY.

Hearing loss in patients with BWS is rare. Several cases of conductive hearing loss due to stapedial and/or malleus fixation have been described. Here we report a 9 year old male with BWS and bilateral severe to profound SNHL. The patient presented with typical BWS features, including macrosomia, omphalocele, macroglossia, hypoglycemia in early infancy, and hemihypertrophy. Diagnosis was confirmed by molecular genetic testing, which showed abnormal methylation of H19 and LIT1 genes, consistent with uniparental disomy of paternal chromosome 11p15 (patUPD). High resolution chromosome karyotyping and EKG were normal. The boy was found to have SNHL at age of 3 years. Parents and a brother are healthy. There is no family history of consanguinity, BWS, deafness or long QT syndrome. The etiologies of SNHL considered included: 1) congenital anomaly of inner ears; 2) connexin 26 mutation(s); 3) aminoglycoside induced ototoxicity due to MTRNR1A1555G mutation; 4) kernicterus or CNS infection; and 5) involvement of KCNQ1 gene. The first three possibilities were ruled out because of normal CT scan of temporal bone and inner ears, and normal connexin 26 sequencing and A1555G mutation analysis. The patient had a history of neonatal jaundice, but not CNS infection. Information regarding the severity of hyperbilirubinemia is not available. Of interest is that the KCNQ1 gene is located in the BWS region, and is maternally expressed in most tissues excluding the heart, where there is biallelic expression. Homozygous mutations of this gene cause Jervell and Lange-Nielsen syndrome (JLNS), a long QT syndrome characterized by SNHL. Recent studies indicate that defective subunit assembly may be responsible for the phenotype of JLNS. Given this mechanism and the lack of reports of SNHL in BWS, it appears that reduced expression of KCNQ1 alone, as expected with patUPD, is unlikely to cause SNHL. Hence the SNHL in our patient could be multifactorial or contributed to by significant neonatal jaundice.

Christians adducted thumbs syndrome with rod-shaped nemaline inclusions. *K. Godbole¹, S. Unger², S. Keating³, W. Halliday^{3, 4}, L. Steele⁴, D. Chitayat^{1,5,6}.* 1) Dept Clinical Genetics, Hosp Sick Children, Toronto, ON, Canada; 2) Department of Pediatrics, Mount Sinai Hospital, Toronto, Ontario, Canada; 3) Laboratory Medicine and Pathobiology Mount Sinai Hospital, Toronto, Ontario, Canada; 4) Department of Pediatric Laboratory Medicine, Hospital for Sick Children, Toronto, ON, Canada; 5) Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, Ontario, Canada; 6) U of T, Toronto, Canada.

Christian syndrome is an autosomal recessive condition consisting of adducted thumbs, camptodactyly, facial dysmorphism, hypotonia, cleft palate and in some craniosynostosis (OMIM #201550).

We present a hypotonic newborn adducted thumbs who was prenatally diagnosed to have isolated, bilateral club feet and 46, XY karyotype. He required resuscitation at birth and remained ventilator dependent until death on the 10th day of life. His weight and length were below the 3rd percentile. He had a myopathic face, overriding cranial sutures with sloping forehead, hypertrichosis, short palpebral fissures with epicanthic folds, long curly eyelashes, simple low set and posteriorly rotated ears, micrognathia with cleft palate and sloping shoulders. He had proximally inserted adducted thumbs, camptodactyly, bilateral club feet with clinodactyly of 5th toes, restricted joint movements and undescended testes with hypoplastic scrotum. There was hypotonia with areflexia. CT scan of his brain was normal. The autopsy showed rod-shaped nemaline inclusions in striated muscles.

This clinical presentation best fits Christians adducted thumbs syndrome. Previously reported muscle biopsy studies in patients with Christians syndrome do not mention this finding except for a couple of reports with myopathic EMG. The finding of nemaline rods can be primary- and Christian syndrome may be one of the clinical manifestations of NRD or secondary and part of Christian syndrome. It is also possible that this patient had two conditions, NRD and Christian syndrome. Further cases may answer these questions.

GOMEZ-LOPEZ-HERNANDEZ SYNDROME: DESCRIPTION OF TWO MORE BRAZILIAN CASES. IS THERE ANY COINCIDENCE? I. GOMY¹, B. HECK¹, C. MARTINELLI², JM. DE PINA-NETO¹, LM. BATISTA¹, MP. CESCATO³, VE. FERRAZ¹. 1) MEDICAL GENETICS, UNIVERSITY OF SÃO PAULO, RIBEIRÃO PRETO, SÃO PAULO, Brazil; 2) ENDOCRINOLOGY PEDIATRICS, UNIVERSITY OF SÃO PAULO, RIBEIRÃO PRETO, SÃO PAULO, BRAZIL; 3) PSYCHIATRY PEDIATRICS, UNIVERSITY OF SÃO PAULO, RIBEIRÃO PRETO, SÃO PAULO, BRAZIL.

Gomez-Lopez-Hernandez syndrome or cerebellotrigeminal dermal dysplasia (MIM#601853) is a recurrent pattern syndrome characterized by neurocutaneous findings that include cerebellar ataxia, parietal alopecia, corneal and facial trigeminal anesthesia, and mental deficiency. All cases reported were sporadic and the etiology remains unknown. However the recurrent-pattern among unrelated patients suggests a common pathogenesis. Brocks et al (2000) reported the eighth case which had been identified as the oldest case and presented psychiatric findings as bipolar disorder and growth hormone deficiency. We now report two more unrelated Brazilian patients, who one of them at the age of 26 years is the new oldest case identified to date. These are the fourth and fifth Brazilian cases of a total of ten that had been reported in the literature since 1979 (Gomez, 1979; Lopez-Hernandez, 1982). Both cases have cranial asymmetry, midface hypoplasia, rhombencephalosynapsis in MRI, biparietal alopecia and mental retardation. The older patient presents findings that had not been described yet such as macrocephaly secondary to obstructive hydrocephalus, spastic paraparesis, seizures, esophageal stenosis secondary to esophageal reflux. Moreover he has growth hormone deficiency but has no trigeminal anesthesia or corneal opacities. Psychiatric evaluation was normal. He was born of consanguineous parents but his ten siblings are normal. The younger patient has corneal opacities and anesthesia, presents bipolar disorder, but has no growth hormone deficiency. Parents are not consanguineous and there are no similar cases in his family. We think that this is not such a rare syndrome and may be likely underdiagnosed, as we identified half of the cases reported to date. The etiology is still not known but the validity of a recurrent-pattern syndrome increases with the more abnormalities found in the condition and the more patients recognized as having the syndrome.

Mosaic trisomy 13: report of one case with abnormal skin pigmentation. *A. Gonzalez-del Angel¹, R. Avendano¹, L.M. Velasco¹, B. Blanco¹, C. Salas¹, R. Ruiz-Maldonado², V. del Castillo¹.* 1) Department of Human Genetics; 2) Department of Dermatology. Instituto Nacional de Pediatría, Mexico City, Mexico.

Mosaic trisomy 13 is a rare entity and as in other cases of chromosomal mosaicism the clinical manifestations are usually milder, although the correlation between phenotype and percentage of trisomic cells has not been established. We report a case of mosaic trisomy 13 with minor dysmorphism, borderline mental development and abnormal skin pigmentary pattern. The patient is a female, 3 years old, pregnancy was uneventful, obtained by spontaneous delivery at 36 weeks of gestation, with a birth weight of 3000g, she remained one month in the intensive care unit with episodes of hypoglycemia and neonatal hyperbilirubinemia. Gesell test was performed, reporting 80% of development in the motor area, 75% in the adaptive area, and 70% in language. At physical examination, weight and height were at 25th centile, cephalic circumference in third centile, bilateral epicanthic folds, strabismus, small ears, normal palate and clinodactyly fifth finger of hands; she had an abnormal pigmentary pattern consisting of hyperpigmented linear and whorly lines, disseminated at trunk and limbs, without midline involvement. Cerebral CT scan, renal US and echocardiography were normal. Blood cytogenetic analysis showed 46, XX/47,XX+13 mosaicism with the abnormal cell line in 36% of analyzed cells, karyotype of light and dark skin showed 9% and 92% of trisomic cells respectively. At least five reports of mosaic trisomy 13 and abnormal pigmentary pattern have been documented and mental retardation is a constant feature; our patient is unique because she has an almost normal development and minimal clinical manifestations, despite the degree of trisomy 13 cell line, which is higher than in previously reported patients. The mosaic trisomy 13 cases described in the literature have a pigmentary cutaneous mosaicism different than the observed Ito's hypomelanosis. This pattern is called phylloid, which resembles an art nouveau picture and oblong macules, that do not follow Blaschko's lines, our patient seems to have this dermatologic manifestation.

Bohring-Opitz syndrome: A new case and review of the literature. *D.K. Grange¹, F.S. Cole²*. 1) Division of Medical Genetics; 2) Newborn Medicine, Department of Pediatrics, Washington University School of Medicine, St Louis, MO.

We report a new case of Bohring-Opitz syndrome, a rare malformation syndrome of unknown etiology. The first patient was reported as a case of Opitz trigonocephaly (C) syndrome. Four strikingly similar cases were described by Bohring (1999), and it was proposed that this could represent the severe end of the spectrum of C syndrome or a distinct disorder. Overlapping features with C syndrome include trigonocephaly, glabellar nevus flammeus, upslanting palpebral fissures, wide alveolar ridges with frenulae, cerebral anomalies, and polydactyly. However, in Bohring-Opitz syndrome, there are more severe anomalies, including exophthalmos, retinal abnormalities, cleft lip and/or palate, flexion deformities of the upper limbs, radial head dislocation, hirsutism, intestinal malrotation, and feeding problems. Many patients died in infancy and had severe developmental disabilities. Our patient was born at 35 wks gestation. She had trigonocephaly with metopic suture synostosis, glabellar nevus flammeus, abundant scalp hair, synophrys, upslanting palpebral fissures, proptotic eyes with ankyloblepharon, small mouth with thick alveolar ridges and prominent midline frenulum, retrognathia, preaxial polydactyly with digitalized thumb, flexion contractures, ulnar deviation of the hands and marked hirsutism. Chromosome analysis and subtelomeric FISH studies were normal. She had a dysplastic pulmonary valve. Brain MRI showed dysplastic, asymmetrically enlarged lateral ventricles. She had malrotation of the intestine. Severe feeding difficulties required gastrostomy tube feedings. Development was delayed. She died at 5 months of age. A total of 11 other patients with Bohring-Opitz syndrome have been reported. In addition, McGaughan (2000) described a similarly affected patient with a duplication of 3p26.3 to 3pter detected by subtelomeric FISH analysis. Greenhalgh (2003) reported two affected siblings, but all other cases have been sporadic. Autosomal recessive inheritance, a new dominant mutation with gonadal mosaicism as the cause of the familial cases, or a cryptic chromosomal rearrangement have all been proposed as potential genetic etiologies for Bohring-Opitz syndrome.

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Malpuech syndrome and ocular-skeletal-abdominal abnormalities (OSA syndrome) are the same: Case Report.
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Mingarelli et al (1996) reported 2 sisters with typical facial features including high arched eyebrows, prominent eyes, ptosis, and malar hypoplasia, diastasis recti secondary to abdominal muscle weakness, lumbosacral spina bifida, scoliosis and radioulnar synostosis. Both were of normal intelligence and stature. Malpuech et al (1983) reported a brother and sister with similar findings plus having heart defects (coarctation, VSD, PDA), cleft lip/palate (Case 1), and caudal appendage. Subsequent reports by Guion-Almeida (1995), Crisponi et al (1999), and Galen-Gomez et al (2004) have confirmed the Malpuech syndrome. I report a 14 year old male who clearly has Malpuech syndrome even including the caudal appendage and PDA, but who also has radioulnar synostosis. Mingarelli's OSA syndrome and Malpuech syndrome are identical facially and share many unique non-facial features, enough in fact to consider them the same autosomal recessive disorder.

Profound Hypertelorism, Midface Prominence, Dental/Enamel Hypoplasia, Prominent/Simple Ears, Osteopenia with Repeated Fractures and Borderline Intelligence in Two Brothers: New Syndrome or An Association of Two Distinct Syndromes? *H. Hamamy*¹, *K. Ajlouni*¹, *A. Teebi*². 1) Genetics, The Jordanian University, Amman, Amman, Jordan; 2) Division of Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto, Ontario.

Profound hypertelorism is very occasionally encountered among patients attending the genetic clinic. The differential diagnosis includes few syndromes namely craniofrontonasal dysplasia and Teebi Hypertelorism Syndrome. We report on two brothers (8 and 10 years old) of first cousin Jordanian Arab parents with the constellation of profound hypertelorism with upslanted eyes on a plane significantly exceeding 180°, midface prominence, peculiar facial appearance, prominent simple ears, dental enamel hypoplasia, osteopenia with repeated fractures and sloping shoulders. Both have mild to moderate sensori-neural hearing loss and both are of borderline intelligence with school difficulties. The father has mild hypertelorism and the family history is negative for severe hypertelorism or repeated fractures. Skeletal survey showed generalized osteopenia. Chromosomes were normal as well as FISH for subtelomeric rearrangements. We think that the constellation of anomalies represent a previously unrecognized syndrome or less likely the association of a unique hypertelorism syndrome and a connective tissue disorder. The inheritance is likely autosomal recessive.

ADOS-G profiles in males with fragile X syndrome. *S.W. Harris¹, B. Goodlin-Jones^{1,2}, S. Nowicki¹, S. Beran¹, D. Hessl^{1,2}, F. Tassone^{1,3}, S. Rogers^{1,2}, R.J. Hagerman^{1,4}.* 1) M.I.N.D. Institute, UC Davis Health System, Sacramento, CA; 2) Department of Psychiatry and Behavioral Sciences, UC Davis Health System, Sacramento, CA; 3) Department of Biochemistry and Molecular Medicine, UC Davis School of Medicine, Davis, CA; 4) Department of Pediatrics, UC Davis Health System, Sacramento, CA.

Stereotypic behaviors associated with autism, including hand flapping and poor eye gaze, have often been described in children with fragile X syndrome (FXS), and some children with FXS are co-morbidly diagnosed with autism. The Autism Diagnostic Observation Schedule Generic (ADOS-G) is a useful tool for assessing autistic features in children and adults with FXS. Each of the four ADOS-G modules includes "presses" designed to elicit behaviors that are associated with autism.

We were interested in looking more closely at the pattern of ADOS classification in boys with FXS, and the specific behaviors that are encompassed in the ADOS algorithm. We assessed 69 males with the full mutation ranging in age from 2 to 18 years (mean=6.8 years, SD 3.7), with an IQ range of 25 to 91 (mean=57, SD=13). The resulting ADOS classifications were no autism 55% (n=38), ASD 16% (n=11), Autism 29% (n=20).

We found that variables such as age at exam and Full Scale IQ (FSIQ) score did not correlate with the overall ADOS classification. FSIQ did, however, have a significant negative correlation with the ADOS classification of the communication domain of the scoring algorithm ($p=.007$). We also found that the rates of the classifications of no autism, ASD, and autism varied by module used for assessment. This may be due to the fact that many of the patients who had lower expressive language skills, and were therefore assessed using module one or two, were those that had autism.

We hope to further investigate specific behaviors captured by the ADOS, as well as the possibility of a specific ADOS profile for individuals with FXS.

Prader-Willi syndrome with multi-suture craniosynostosis: Report of two cases. *R. Klatt¹, L. Velsher², S. Dyack³, P. Ray⁴, L. Steele⁴, T. Barozzino⁵, M. Sgro⁵, A. Teebi¹, D. Chitayat^{1,6}.* 1) Division of Clinical & Metabolic Genetics, Dept. of Paediatrics, The Hospital for Sick Children, Toronto, ON, Canada; 2) Genetics Program, North York General Hospital, Toronto, ON, Canada; 3) Division of Medical Genetics, Dept. of Paediatrics, IWK Health Centre, Halifax, NS, Canada; 4) Division of Molecular Genetics, Dept. of Paediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, ON, Canada; 5) St. Michael's Hospital, Toronto, ON, Canada; 6) Prenatal Diagnosis & Medical Genetics Program, Mount Sinai Hospital, Toronto, ON, Canada.

Prader-Willi syndrome (PWS) is a genetic condition that presents early in life with hypotonia, feeding difficulties, failure to thrive, and characteristic facial features. In early childhood, the condition typically involves obesity and developmental delay as well. The PWS critical region is 15q11-13. We report on two unrelated patients with PWS and craniosynostosis confirmed by imaging studies. Patient 1 was born to healthy non-consanguineous parents of Pakistani descent. The pregnancy was complicated by poor fetal movements. Severe hypotonia and dysmorphic features, including abnormal head shape, were noted at birth. Chromosome analysis showed 46, XX and FISH analysis using probes for SNRPN and D15S10 showed a deletion of 15q11-13. Abnormal methylation was demonstrated. DNA analysis for FGFR2, FGFR3, and TWIST was negative. A skull x-ray and CT scan confirmed bicoronal synostosis. Patient 2 was born to healthy consanguineous parents of Sri Lankan descent. Poor feeding and hypotonia were noted neonatally. Chromosome analysis was 46,XX and there was abnormal methylation of the SNRPN gene. DNA analysis for FGFR3 was negative. Abnormal head shape was initially noticed at 5 months of age. A CT scan confirmed bicoronal and metopic synostosis. Review of the literature revealed one prior case of PWS with craniosynostosis, but this was not confirmed by CT scan or x-ray. Therefore, these are the first two cases of documented craniosynostosis in association with PWS. We hypothesize that dysfunction of the PWS critical region can result in the disturbance of one or more genes associated with craniosynostosis.

OEIS complex with del(3)(q13.2.q21). *R. Kosaki¹, T. Okuyama¹, N. Kawashima², T. Honna², K. Ueoka³, K. Kosaki⁴.*
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Most cases of cloacal extrophy occur sporadically, but there have been several reports of recurrence in siblings, including monozygotic twins, suggesting a genetic contribution to the pathogenesis of this condition. A case of cloacal extrophy with a de novo 9q34.1-qter deletion was recently reported. The case represented the first report of cloacal extrophy accompanied by an unbalanced chromosomal defect. Here, we report a patient with cloacal extrophy who exhibited a de novo deletion at another chromosomal location, 3q13.2.-3q21. [Case report] The male patient was born at 41 weeks to a non-consanguineous Japanese couple after an uneventful pregnancy without teratogen exposure. The boy, who weighed 3018 g, exhibited cloacal extrophy with omphalocele, ileal atresia, and an imperforate anus. A scrotal fold was present, but no penile structure was visible. Dysmorphic features included low-set ears, midface hypoplasia, hypertelorism with epicanthal folds, a high arched palate, and a pointed chin. X-ray studies revealed symphysis pubis diastasis, a malformed sacrum, spina bifida, and butterfly vertebrae of the lower thoracic region. He also exhibited tethered cords and skin-covered lipomyeloceles. The patient was diagnosed as having (omphalocele-exstrophy-imperforate anus-spinal defects (OEIS) complex. On the second day after birth, the omphalocele and anterior wall of the bladder were surgically repaired. At the age of two months, the cloaca was divided into a colon and a bladder, and the anterior wall of the bladder and the abdominal wall were repaired. G-banding chromosome analysis revealed a 46,XY,del(3)(q13.2.q21) karyotype. The parents had normal karyotype. The present description of a 3q interstitial deletion and the report describing a 9q terminal deletion give further credence to the concept that genetic defects may be responsible for the pathogenesis of cloacal extrophy / OEIS complex and indicate potential loci for the putative genes.

Textbook Features of Saethre-Chotzen Syndrome in Six Members of a Previously Undiagnosed Family: An Exciting Day in the Office, a Reason to TWIST and Shout. *D. Kostiner¹, K. Crow¹, C. Miranda¹, M. Brock², J. Israel².* 1) Dept Genetics, Kaiser Permanente, Portland, OR; 2) Dept ENT.

Saethre-Chotzen syndrome (SCS) is an autosomal dominant condition that affects ~1/37,000. No single feature is obligatory, but typical features include craniosynostosis, low frontal hairline, hypertelorism, ptosis, lacrimal duct defects, small ears, deviated septum, maxillary hypoplasia, narrow palate, partial 2/3-finger syndactyly, and brachydactyly. We present 6 relatives with unmistakable features of SCS to illustrate the consistency of features within the family and to explore the possible genotype-phenotype correlation of the causative mutation. TWIST1 gene analysis is currently pending in the proband. 1) LH, age 18, has hypertelorism, dacryostenosis, small ears, recurrent otitis, surgeries for broad nose and deviated septum, high/narrow palate, partial 2/3-finger syndactyly, transverse palmar creases, and brachydactyly. 2) Her brother, 11, has ear tubes, deviated septum, high/narrow palate, speech problems, and partial 2/3-finger syndactyly. 3) Her father, 43, had surgeries for ptosis, deviated septum, and dental crowding, partial 2/3-finger syndactyly, and premature graying. 4) Her uncle, 46, had surgery for ptosis and deviated septum. 5) Her grandfather, 70s, has severe L-sided ptosis, prominent nose, and partial 2/3-finger syndactyly. 6) Her great grandmother, deceased at 80, had short stature, exophthalmos, hypertelorism, and possible craniosynostosis. Most cases of SCS are due to changes in the TWIST1 gene on chromosome locus 7p21. TWIST1 is a basic helix-loop-helix (bHLH) transcription factor that apparently helps specify and maintain cell identity. Genotype-phenotype correlations for different mutations within exon 1 have not been observed, i.e. any mutation that alters the sequence seems to cause loss of functional TWIST1 protein. Complete TWIST1 deletions, translocations, inversions, and ring 7 may additionally cause atypical features and delay. Some patients with SCS-like features have the FGFR3 P250R mutation associated with Muenke syndrome. Confirming a mutation in this family might help contribute to the understanding of the Saethre-Chotzen phenotype.

Natural history of Sotos syndrome with submicroscopic deletion of 5q35 including NSD1. *K. Kurosawa¹, T. Yamamoto¹, M. Masuno², K. Imaizumi¹, Y. Kuroki².* 1) Clinical Research Institute, Kanagawa Children's Med Ctr, Yokohama, Japan; 2) Kawasaki University of Medical Welfare, Kurashiki, Japan.

Sotos syndrome is an autosomal dominant overgrowth syndrome with characteristic craniofacial dysmorphic features and various degrees of mental retardation. Two thirds of Japanese patients with Sotos syndrome had a common 2.2 Mb deletion involving the entire NSD1 gene, and subset of of patients had NSD1 point mutations. The clinical features are classified according to the dosage effects of NSD1 gene. The patients with submicroscopic deletion of 5q35 including NSD1 gene show more severe phenotype than those of point mutation type. We have evaluated 14 (10 boys and 4 girls) individuals with Sotos syndrome of submicroscopic deletion. We review the clinical features, the spectrum of associated abnormalities, and management of complications according to medical records. The diagnosis is based on the clinical features and cytogenetic analysis including the standard GTG and FISH analysis with RP1-118M12 PAC clone, involving the NSD1 gene. The mean birth weight was 3310 g, length 49.5 cm. Overgrowth became less remarkable at later age, but macrocephay was prominent according to growth. 8 cases (57%) had seizures and 6 were medicated with antiepileptic drugs. CNS abnormalities such as ventricular dilatation, hypoplastic corpus callosum, and multiple ependymoma was noed in 9 cases. Infantile hypotonia and feeding difficulty was the major problems in the neonatal period. Delayed developmental milestones were evident from infantile periods in all cases. Most cases had moderate to severe mental retardation. Congenital heart disease occurred in 6 cases and was important determinant of survival. The patterns of CHD was complex with pulmonary valve atresia and tetralogy of Fallot. Urogenital problems including vesicouretero-reflux, hydroenephrosis, and hypospadias occurred in 8 (57%). The information of natural history of Sotos syndrome with NSD1 deletion was suggested to be important for management and caring the disorder.

Two cases of chromosome 8p23 deletion with different clinical presentation. *L. L. Fishman¹, G. Maegawa¹, R. Babul-Hirji¹, D. Chitayat^{1,2}, A. Teebi¹.* 1) Div of Clin & Metab. Genetics, Hospital for Sick Children, Toronto, ON, Canada; 2) Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, U of T, Toronto, Ontario, Canada.

We report the clinical findings of two patients with a terminal deletion of the short arm of chromosome 8. Case 1: 14 year-old boy with moderate developmental delay and some aggressiveness outburst. He was born to healthy parents with a non-contributory family history. Pregnancy was unremarkable and delivery was normal without any perinatal complication. Physical examination showed microcephaly, mild scoliosis and dysmorphic features such as a low nuchal hair line and two hair-whorls close to vertex. He had a dysphonic voice. His neurological exam was normal except from his cognitive dysfunction. The chromosome showed 46, XY, del(8)(p23.1). Parents karyotypes were normal. Case 2: 11 year-old boy who presented with developmental delay and congenital heart disease. Parents were healthy and family history was unremarkable. Pregnancy was uncomplicated and he was born by induced vaginal delivery at 32 weeks gestation. He had some cyanotic spells soon after birth and an echocardiography revealed small VSD, ASD and pulmonary stenosis. He presented delay in achieving his developmental milestones. On physical examination, his OFC was 51.7 cm (- 1SD). He had dysmorphic features such as brachycephaly, flat occiput, low nuchal hairline with webbed neck, bitemporal narrowing, droopy eyelids and hypoplasia of malar areas. He had bilateral inverted nipples, systolic ejection murmur. He had digitalized thumbs and pes planovalgus bilaterally. His neurological exam showed a decreased muscle bulk and tone. His chromosome showed 46, XY, del(8)(p23.1). The parents chromosomes were normal. These two cases represent the clinical variability of the 8p23 deletion syndrome. The cardiac malformations are present in up to 2/3 of patients. The typical destructive and aggressive behavior is also characteristic of this syndrome. However the facial dysmorphic features could be mild or even absent which could delay the diagnosis of a chromosomal abnormality on the differential work-up.

Mosaic Duplication of 9q11qter: A case report and review of published cases for the dup(9q) syndrome. *A.H.M. Lai¹, M.H. Yong², L. Knight², S.H. Teo¹, I.S.L. Ng¹.* 1) Genetics Service, Department of Paediatric Medicine, KK Women's and Children's Hospital, Singapore; 2) Cytogenetics, KK Women's and Children's Hospital, Singapore.

Partial trisomy 9q is a rare chromosomal abnormality that results in a variable clinical phenotype. Clinical features reported include deep-set eyes, microdolichocephaly, a beaked nose, and microretrognathia. We present a female infant with an unbalanced mosaic karyotype: 46,XX,der(14)t(9;14)(q11;p11.1)[3]/46,XX[57] resulting in duplication of 9q11 to 9qter in 3 of the 60 cells analysed. She has a high forehead, short palpebral fissures, bilateral ptosis, a small mouth, microretrognathia, a right single palmar crease, a ventricular septal defect, laryngomalacia, hypothyroidism, hearing impairment, bilateral visual impairment and severe global developmental delay. This patient is compared with reported cases with pure partial dup(9q). Some features described in almost all the reported cases, such as deep-set eyes, beaked nose, long fingers and stiff joints, are not observed in our patient.

Possible autosomal recessive inheritance of Cornelia de Lange -like syndrome. *A.F. Lewanda, M.E. Nunes.* Inova Fairfax Hosp Children, Fairfax, VA.

Cornelia de Lange syndrome (CdLS) is a dominant disorder characterized by facial dysmorphism and micromelia, recently found to be caused by *NIPBL* gene mutations. We report three patients from two unrelated, consanguineous families who have CdLS features. This may represent recessive inheritance. Parents in family 1 are second cousins from Somalia. Their first child was seen as a newborn with microcephaly, synophrys, deep set eyes, downturned mouth, hypoplastic nipples, small distal phalanges, 2-3 toe syndactyly, hypertonicity and irritability. By two years of age, she was at the 5th percentile for height and weight, and remained microcephalic. She had also developed GE reflux, long curly eyelashes, bilateral radial head dislocation, and hypoplastic proximal radii. Her hand length was average for a 6 month-old, and foot length for a 1 year-old. Karyotype and methylation studies for PWS were normal. Their next child was SGA and microcephalic, hirsute, with synophrys, long eyelashes, anteverted nares, and tapered fingers without gross reduction. Cardiac evaluation revealed a dysplastic pulmonary valve with stenosis. Karyotype was normal. Parents in family 2 are first cousins from the Middle East. Their newborn (patient 3) had microcephaly, hoarse cry, and unilateral clubfoot with limb atrophy. By four months, she developed microbrachycephaly, and severe GE reflux requiring Nissen fundoplication. Examination revealed growth parameters at the fifth percentile (head notably smaller), synophrys, long eyelashes, prominent columella, anteverted nares, retrognathia, hypoplastic nipples, short metacarpals with single palmar crease, atrophy of the right leg, and hirsutism. Cry remained low-pitched. Karyotype was normal. In both families, parents did not show features of CdLS. Features of patients 1 and 3 became more obvious with time. While only dominant or sporadic inheritance have previously been ascribed to CdL or CdL-like syndromes, our patients suggest the possibility of a recessive CdL-like syndrome. This would be distinct from published cases in which the mutation identified in multiple affected siblings has not been found in parents. We are pursuing *NIPBL* mutation testing in these families to distinguish between these hypotheses.

FURTHER DELINEATION OF THE GUADALAJARA CAMPTODACTYLY TYPE II (GSTII). *G. Lopez-Cardona*^{1,6}, *D. Garcia-Cruz*^{2,6}, *N.O. Davalos*^{2,6}, *B. Patio-Garcia*^{2,6}, *L.X. Rodriguez-Rojas*^{2,6}, *M.X. Ruiz*⁷, *M.O. Garcia-Cruz*⁵, *Z. Nazar*³, *I.P. Davalos*^{2,6}, *W.E. Bitar-Alatorre*⁴, *J. Sanchez-Corona*^{1,6}, *J.M. Cantu*^{2,6}. 1) Division de Medicina Molecular, Centro de Investigacion biomedica, Guadalajara, Jalisco, Mexico; 2) Division de Genetica, Centro de Investigacion Biomedica, Guadalajara, Jalisco, Mexico; 3) Servicio de Radiologia, Hospital de Especialidades, CMNO, IMSS, Guadalajara, Mexico; 4) Servicio de Ortopedia, Hospital de Especialidades, CMNO, IMSS, Guadalajara, Mexico; 5) Servicio de Labio y Paladar Hendido HGR46, IMSS, Guadalajara, Mexico; 6) Doctorado en Genetica Humana, Universidad de Guadalajara; Guadalajara, Jalisco; Mexico; 7) Servicio de Genetica, Hospital Metropolitano, Quito; Ecuador.

Our purpose is to describe the clinical and radiological features in a new case of GCSTII and to propose a further delineation of this syndrome. We describe a 38 years old woman with low-set hair, unusual facies, small pinnae with ample conchae, short and wide neck with limited lateral and flexion movements; camptodactyly, limitation of flexion movements at the knees and extension at the ankles, skin syndactyly and clinodactyly of the 4 and 5 toes. X-ray findings: brachycephaly, scoliosis, cuboid-shaped vertebral bodies, camptodactyly of 4 and 5 fingers with subdislocation of the 1 finger in right hand; flexion contracture of the 3 to 5 prominent metacarpophalangeal joint of the 1 finger in the left and dysplastic distal epiphysis of the proximal phalanx, compacted carpal bones on both hands. Congenital hip dislocation, acetabular hypoplasia, hypoplastic iliac bones and ischiopubic branches, hypoplasia of left femoral neck and slender femoral, tibial and fibular bones; bilateral hypoplasia and upper dislocation of patellae. Feet: bilateral hypertrophy of 1st rays and prominent talus. The propositas parents are healthy and consanguineous in accordance with autosomal recessive inheritance suggested for GCSTII (MIM211920) by Cantu et al 1985.

Trisomy 17p10-p12 due to mosaic supernumerary marker chromosome: delineation of molecular breakpoints and clinical phenotype and comparison to other proximal 17p segmental duplications. *J.R. Lupski^{1,2,5,6}, S.A. Yatsenko^{1,5}, D. Treadwell-Deering^{2,3,5,6}, K. Krull^{2,3,5,6}, D. Glaze^{2,4,5,6}, M. Horz^{1,5}, P. Stankiewicz^{1,5}, L. Potocki^{1,5,6}.*
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The unstable, gene-rich 17p11.2p12 region is associated with a wide variety of structural chromosomal aberrations. Supernumerary marker chromosome 17 (SMC17) comprises a subset of these, and the majority of SMC17s often utilize the same substrates for recombination as the common 17p11.2 and 17p12 rearrangements. We report a 9-year-old girl with a *de novo* mosaic SMC17. The der(17) encompasses genetic material from 17p10-p11.2 and is present in 97% of peripheral blood lymphocytes and 78% of buccal cells. The patient has few features similar to individuals with duplication 17p11.2 including mental retardation, language impairment and sleep disturbances but has normal growth, and no structural abnormalities of the heart, kidneys or brain. She has no significant behavioral abnormalities or dysmorphic features. Molecular analyses determined that the der(17) contains *RAI1* but not *PMP22*. One breakpoint was identified within the centromere and the second breakpoint maps within the distal SMS-REP (Smith-Magenis syndrome low-copy repeat, LCR). We have recently characterized the breakpoints of three other marker chromosomes originating from the proximal short arm of chromosome 17. In all three cases, one breakpoint maps within the centromere and in two cases the second breakpoint maps within a LCR, suggesting that genome architecture may play a significant role in the formation of marker chromosomes. Herein, we present the cytogenetic, molecular, and clinical data of this patient and compare our results with those of patients with dup(17)(p11.2p11.2) syndrome and other SMC17 patients.

Deletion 2q37.1 - an emerging phenotype. *J.J. MacKenzie^{1,3}, G. Davies², K. Harrison^{1,3}*. 1) Department of Pediatrics, Queen's University, Kingston, ON, Canada; 2) Department of Obstetrics and Gynecology, Queen's University, Kingston, ON, Canada; 3) Department of Pathology, Queen's University, Kingston, ON, Canada.

We report a new case of an individual with the karyotype 46,XX,del(2)(q37.1)[20]/46,XX[10] initially detected at an amniocentesis prompted by a large pleural effusion identified on ultrasound at 18 weeks gestation. We now have 6.5 years of follow up data on this child and have compared her findings with other cases in the literature. Our patient's clinical features include: dolicocephaly, fine hair, short forehead, frontal bossing, hypertelorism, short nose with an overturned tip, a hypoplastic malar area, long philtrum, thin upper lip, arched palate, low set ears with small attached lobules, short neck, broad chest, arachnodactyly with transverse palmar creases, gibbus deformity, lax joints and mild hypotonia. She is also affected with developmental delay and carries a formal diagnosis of autism.

A review of other cases has allowed us to determine similarities in the phenotype which are starting to emerge including: sparse hair, frontal bossing, hypertelorism, low set ears, arched or cleft palate, long philtrum, thin upper lip, short neck, arachnodactyly, and psychomotor retardation. Recently, two cases have been reported in a series of individuals with autism and a review of the literature describes perseverative behavior in other cases which is consistent with the diagnosis in our patient.

At the time of prenatal diagnosis, limited information was available, regarding this deletion, to provide to the family. Further delineation of the phenotype in persons with a 2q37.1 deletion has the potential to improve detection using molecular cytogenetic techniques in those who do not have a microscopically visible change. This will provide us the opportunity to further define the phenotype and to correlate the clinical features with specific genes in this region. We suggest that a 2q37.1 deletion should be considered in individuals with the described features and a careful clinical exam for these should be done in patients presenting with signs of autism.

Cutis marmorata telangiectatica congenita and gastroschisis: an unusual association. *L. Martelli, I. Gomy, C. Lourenco, L. Batista, SE. Hansing, DRD. Carvalho, J. Souza, JM. Pina-Neto.* Dept Genetics-FMRP, Univ Sao Paulo, Ribeirao Preto SP, SP, Brazil.

Cutis marmorata telangiectatica congenita (CMTC) is a localized or widespread vascular anomaly, usually present at birth, and characterized by a persistent reticular vascular pattern with a marble-like bluish or deep purple appearance. Atrophy or hypertrophy of the underlying tissue are commonly reported features. In addition, CMTC is often reported in association with a variety of other congenital anomalies, including but not limited to undergrowth or overgrowth of an involved extremity. The first report of CMTC occurred in 1922 by Van Lohuizen who described a child with livedo reticularis, telangiectases, and superficial ulceration. The pathogenesis of CMTC remains unclear, and the cause may be multifactorial. Most cases occur sporadically, although rare cases occur in families. We report a case of a neonate, born at 39 weeks, the first child of non consanguineous marriage, whose obstetric ultrasound has revealed gastroschisis. At birth, physical examination showed gastroschisis, criptorchidia and slightly bluish red, flat, reticulated pattern over the trunk and legs, more marked on the left side than the right side. The differential diagnosis included Adams-Oliver syndrome. To our knowledge, this is the first description of CMTC and gastroschisis in the same patient. Sponsored by: FAEPA.

Lobodontia - further characterization of a rare dominantly inherited disorder of dental morphogenesis. *F.A. McKenzie¹, E. Mahoney²*. 1) Hunter Genetics, Newcastle, NSW, Australia; 2) Department of Paediatric Dentistry, Westmead Hospital, Sydney, Australia.

Lobodontia is a rare autosomal dominant disorder of dental morphogenesis. It is characterized by delayed eruption and prolonged retention of the primary dentition and generalised morphological abnormalities, including multitubercular molar crowns and conical molar roots, small crown size, pointed buccal cusps of premolars and canines and peg- or shovel-shaped incisors. Hypodontia and tooth invagination is also described. There are only three cases previously reported in the medical literature. We present a fourth family with this disorder and further delineate the associated features. The molecular basis for this disorder is not known but it is postulated that an abnormality of dental development occurs after tooth type has been determined, but at an early stage of morphological differentiation that affects all tooth types of the permanent dentition.

Bilateral microtia, absent external auditory meati, Mondini type malformation, duodenal atresia, thyroid hemiplasia and biliary atresia. A New syndrome. *P. McNamara¹, G. Maegawa², H. White¹, M. Thomas⁵, G. Taylor³, J. Kim¹, P. Kim⁴, D. Chitayat^{2,5}.* 1) Neonatology; 2) Div of Clinical and Metabol. Genetics; 3) Dept of Pathology; 4) Div of Paediatric Surgery, Hosp. for Sick Children; 5) Prenatal Diagnosis and Medical Genetics Prog., MSH, U of T, Toronto, Ontario, Canada.

We present a previously apparently undescribed syndrome with a unique pattern of malformations. A female was born at 36 wks GA with multiple abnormalities including IUGR, coarse facial features, bilateral microtia with absence external auditory meati, duodenal atresia (DA), intestinal malrotation and anterior displacement of the anus. Parents were healthy and non-consanguineous. The 1st pregnancy was terminated for sirenomelia and the 2nd resulted in a healthy son. The pregnancy was initially uncomplicated. MSS was done at 20.3 weeks and showed an increased risk for Down syndrome (1:356). Amniocentesis showed 46, XX. A 3rd trimester U/S showed IUGR and double bubble indicative of DA. Her bwt was 1550 g (<3rd), blt was 45 cm (10th) and OFC was 30.5 cm (<2nd). At birth, microcephaly and bilateral microtia with absence of the external auditory meati were noted. A lt. eye esotropia was evident. There was an asymmetric crying with the mouth deviating to the rt. side, micrognathia and anterior displacement of the anus. Surgery showed complete DA with blind-ended jejunum in lt.-lower quadrant with a long pedicle and intestinal malrotation with Ladd's bands. Cholescintigraphy revealed no evidence of bile drainage suggesting biliary atresia. The TSH was elevated and the thyroid scintigraphy revealed poor lt. lobe uptake. The neck U/S confirmed the hemiplasia of the left thyroid lobe. The brain MRI/MRS were normal. The CT-scan for petrous bone showed bilateral Mondini type deformity, bilateral absence of external auditory canals and internal fused ossicles. The association of DA with intra and extra-hepatic biliary atresia has been reported in the past. However, the association with ear anomalies, thyroid and gastrointestinal malformations has not been reported. We thus suggest that this constellation is a hitherto previously undescribed new syndrome.

Hermansky Pudlak Syndrome in two African-American brothers. *M.A. Merideth^{1,2}, S.E. Sparks¹, K.J. O'Brien^{1,2}, W.A. Gahl¹.* 1) Section on Human Biochemical Genetics, Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland; 2) Intramural Office of Rare Diseases, Office of the Director, National Institutes of Health, Bethesda, Maryland.

Hermansky Pudlak Syndrome (HPS) is an autosomal recessive disorder characterized by oculocutaneous albinism, a bleeding disorder secondary to a platelet storage pool deficiency, and in some patients, lysosomal accumulation of ceroid lipofuscin. A subset of patients develops fatal pulmonary fibrosis and granulomatous colitis. There exist seven subtypes of HPS, each due to mutations in one of seven different genes, whose functions are thought to involve vesicle formation and trafficking. HPS has been identified primarily in patients of Puerto Rican, Northern European and Japanese descent, although affected individuals have been ascertained in nearly all ethnic groups. We report HPS in two African-American brothers, diagnosed at ages twelve and thirteen years by electron microscopy showing absence of platelet dense bodies. Both boys had been diagnosed with oculocutaneous albinism at birth. They also had excessive bruising that was apparent in the two years prior to diagnosis of HPS. The younger brother had a gastrointestinal bleeding episode immediately prior to his diagnosis of HPS at the age of twelve years. The elder brother has nystagmus, iris transillumination and a visual acuity of 20/160 bilaterally. The younger brother also has nystagmus and iris transillumination, and a visual acuity of 20/160 on the right and 20/125 on the left. Laboratory evaluations are currently underway to determine the HPS subtypes of these two patients. To our knowledge this is the first diagnosis of HPS in African-American patients. The diagnosis of HPS should be looked for in this and all ethnic groups with oculocutaneous albinism, and not just among Puerto Ricans.

A New Association with Ectrodactyly-Synechia of the Labia Minora-Coloboma Parpebral and Palsy Facial. J.

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Ectrodactyly, is a congenital limb malformation, characterized by a deep median cleft of the hand and/or foot due to the absence of the central rays and may occur as an isolated entity or as part of a syndrome. More than 50 syndromes and associations are distinguished. The ectrodactyly can be seen with associated malformations, cleft lip/palate, polydactyly, triphalangeal thumbs, ectodermal dysplasia, keratosis, urinary tract malformations, cutis aplasia congenita, macular dystrophy, sensorineural hearing loss, microcephaly, microphthalmia, sagittal craniostenosis, mental retardation, spina bifida, diaphragm defects, congenital heart disease, tibial hypoplasia, fibular hypoplasia, holoprosencephaly, micropenis, cryptorchidism and transverse vaginal septum. A large number of human gene defects can cause ectrodactyly. The most common mode of inheritance is autosomal-dominant, the autosomal-recessive and X-linked forms occur more rarely and other cases of ectrodactyly are caused by chromosomal deletions and duplications. We report on a female infant 3 years old, product of the fifty pregnancy. She was delivered vaginally at 37 weeks of gestation without complications. Her parents are not consanguineous. The positive findings found on the physical examination were: prominent forehead, coloboma parpebral, anteverted nostrils, wide spaced central incisors, facial palsy, hypopigmented skin, ectrodactyly right of the hand and foot, clinodactyly of fifth finger bilateral, syndactyly and synechia of the labia minora. R-banded chromosomes in peripheral lymphocytes were normal with no evidence of chromosome instability or other anomalies. The result of the karyotype was 46, XX. Here we report a case with additional phenotypic manifestations mainly in genitalia, limbs and eyes. This report supports the existence of a new association of ectrodactyly with malformations in genitalia and eyes.

Cornelia de Lange syndrome and congenital glaucoma: a case report and review of the literature. *S. Nallasamy*¹, *T.L. Young*¹, *D. Yaeger*², *J. McCallum*², *M. Kaur*², *I.D. Krantz*². 1) Ophthalmology and Genetics, Children's Hospital of Philadelphia and University of Pennsylvania School of Medicine, Philadelphia, PA; 2) Human Genetics and Molecular Biology, Children's Hospital of Philadelphia and University of Pennsylvania School of Medicine, Philadelphia, PA.

Cornelia de Lange syndrome (CdLS) is a multi-system congenital disorder that is characterized by dysmorphic facial features, hirsutism, limb abnormalities, mental retardation, growth retardation, gastroesophageal dysfunction, cardiac, ophthalmologic and genitourinary anomalies. Recently, it was discovered that mutations in the *NIPBL* gene on chromosome 5p13 cause CdLS. The most common eye findings in CdLS include: hypertrichosis of the eyebrows; synophrys; long, arcuate eyelashes; telecanthus; myopia; ptosis; nystagmus; and chronic conjunctivitis - often due to nasolacrimal duct obstruction (NLDO). To our knowledge, there has been one reported case of CdLS with glaucoma in a newborn with aniridia presenting with profound buphthalmos. Herein, we present a new case of a male infant with CdLS and congenital glaucoma. The patient was diagnosed with CdLS as a neonate, at which time his eyes were unremarkable. His first complete ophthalmologic exam at 13 months of age demonstrated bilateral NLDO, ptosis, significant myopia, elevated intra-ocular pressures and bilateral optic nerve cupping indicative of glaucoma, and longer than average axial eye lengths. The patient underwent a trabeculotomy in both eyes for congenital glaucoma. Probing and irrigation for NLDO was unsuccessfully attempted and the patient will likely require creation of new nasolacrimal duct conduits. This case illustrates the severity of the eye findings that may be associated with CdLS, and reports a novel presentation of congenital glaucoma without apparent anterior segment structural defects. Interestingly, this patient has a previously unreported deletion, 5721delTGAAA, in exon 31 of *NIPBL*. Towards a better etiologic understanding of the extensive and variable ophthalmologic involvement in CdLS, we are currently gathering ophthalmologic and genotypic data from a large cohort of individuals with CdLS to evaluate for genotype-phenotype correlations.

AXIAL MESODERMAL DYSPLASIA COMPLEX (AMCD). REPORT OF A FIRST CASE IN MEXICO AND REVIEW OF THE LITERATURE. *J. Nastasi, JM. Mantilla, LE. Becerra, M. Díaz, ML. Ramírez, LE. Figuera.* Genética, CIBO-IMSS, Guadalajara, Jalisco, México.

Classically, it has been suggested that Goldenhar spectrum or related syndromes, and caudal regression syndrome are included within axial mesodermal dysplasia complex. Characteristics finding of this condition are: craniofacial abnormalities such as asymmetrical face, preauricular tags, dysplastic ears, congenital heart defects, as well as spinal, gastrointestinal and urogenital malformations. AMCD results from failure of mesodermal cell migration, at early blastogenesis. The defect pattern in this developmental field depends on the time when the causal agent was present, for instance gestational diabetes. The proposita, 11 month old, was born from unrelated parents, 35 years old both. There is no family history of congenital malformations. Three healthy sibs and the mother had two miscarriages. During the pregnancy the mother had gestational diabetes, the child was born after 38 weeks of gestation, she was obtained by vaginal delivery. At birth, her weight was 2.075 g, height 35 cm, and occipital-frontal circumference 31 cm. Multiples malformations were noted at birth and physical examination revealed: abundant hair with frontal low hairline, slant up palpebral fissures, high and wide nasal bridge, anteverted nares, short filtrum, microretrognathia, bilateral multiple preauricular tags in the trago-oral line, short neck, thorax, and abdomen without alterations, genitalia with hipoplastic mayor labia. Spine with an excrescence of dorso-lumbar column. Upper limb without apparent malformations. Lower limbs hipoplastics, bilateral tags on the acetabular region, pterigium popliteal, and club foot bilateral. RX: It was observed lumbosacra spine agenesis, and pelvis fused and hipoplastic. Abdominal ultrasonograms normal. Karyotype was 46,XX. This is the first reported case in Mexico, in the present case, the gestational diabetes could be the agent causing this complex. It has been proposed that AMDC is compound by a spectrum of diverse entities, including Goldenhar syndrome, VACTERL association, caudal regression syndrome and Klippel Feil syndrome. All of them display overlapping signs, then they can be manifestations of the same complex.

COACH syndrome: description of 3 new cases and proposed minimal diagnostic criteria. *M.A. Parisi¹, M. Gentile², L. Finn³, I.A. Glass¹, W.B. Dobyns⁴.* 1) Dept Ped/Div Gen & Develop, Children's Hosp & Reg Med Ctr, Seattle, WA; 2) Dept Med Genet, IRCCS, Castellana Grotte, Italy; 3) Dept Pathology, Children's Hosp & Reg Med Ctr, Seattle, WA; 4) Dept Human Genet, Univ of Chicago, Chicago, IL.

COACH syndrome is a rare autosomal recessive condition characterized by Cerebellar vermis hypoplasia (CVH), Oligophrenia (mental retardation), Ataxia, Colobomas, and Hepatic fibrosis. Renal involvement may include juvenile nephronophthisis. In some cases, the molar tooth sign (MTS) has been identified on MRI; this hindbrain malformation consists of CVH, a deep interpeduncular fossa, and elongated superior cerebellar peduncles. The MTS is a key radiologic sign for Joubert syndrome (JS; hypotonia, developmental delay, breathing abnormalities, oculomotor apraxia, and variable features such as renal disease and colobomas). Here we describe three new cases of COACH syndrome and propose minimal diagnostic criteria. Two of the subjects have colobomas, and two exhibit a classic MTS. A total of 30 cases are now reported, including 9 sets of affected siblings. Thirteen were male (47%). The MTS was identified in 10 subjects with suitable imaging. Colobomas were present in 63%, and renal involvement in 60%. The age of onset of hepatic involvement was variable, ranging from 6 months to 46 years. For 14 subjects, hepatic enlargement was the first indication of liver dysfunction; 4 had abnormal liver function tests, and 4 presented with acute gastrointestinal bleeding. Several children responded to ursodeoxycholic acid, and three received liver transplants. Because of the significant clinical overlap between COACH and Joubert syndromes, COACH can be considered a JS-related disorder. We propose minimal diagnostic criteria for COACH syndrome: CVH (particularly the MTS), mental retardation, and hepatic fibrosis. Ocular colobomas are not necessary for the diagnosis, and renal involvement is variable. Because of the difficulty in predicting which children with JS will develop hepatic fibrosis or renal cystic disease, we recommend routine screening for these complications in children with JS, as early identification and treatment of liver and kidney disease may slow their progression.

Autism in Angelman Syndrome. *S.U. Peters¹, A.L. Beauder², N.S. Madduri¹, T. Sahoo², C.A. Bacino².* 1) Depts. of Pediatrics & 2) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX. 77030.

Angelman syndrome (AS) is a neurodevelopmental disorder characterized by severe mental retardation, ataxia, and a happy/sociable disposition. Maternally inherited chromosome 15q abnormalities, such as duplications, within the AS critical region result in autistic symptomatology, implying a role for the UBE3A gene in autism etiology. This study examined the prevalence of autism in AS in 19 children representing three known molecular classes of AS (deletion positive, UPD, and UBE3A mutations). Children were studied over the course of 1 year. Eight of 19 children (42%) met criteria for autism according to the Autism Diagnostic Observation Schedule. Seven of 16 children who were deletion positive met criteria for autism, and 1 of 2 children with UPD met criteria for autism. The one child with a UBE3A gene mutation did not meet criteria for autism. There were no differences in autism diagnosis as a result of chronological age. Parents of children who were diagnosed with autism according to the ADOS-G were administered the Autism Diagnostic Interview Revised. Data from the ADI-R were convergent with data from the ADOS-G in all cases. Children with co-morbid autism and AS scored significantly lower on measures of language ($p < .01$), adaptive behavior (socialization skills; $p < .05$), and cognition ($p < .01$), and demonstrated a slower rate of improvement over the course of the study. All 8 children who initially met criteria for autism continued to meet criteria for autism after an additional year of follow-up, suggesting that the diagnosis of co-morbid autism could not be solely attributable to developmental delays because symptoms of autism did not decrease in severity over time. Children with co-morbid autism specifically demonstrated deficits in communication and socialization that mirror those observed in children with idiopathic autism. Further analyses are underway to determine how 15q11-q13 deletion size and location are related to a diagnosis of autism within our group of deletion positive patients. This study highlights the phenotypic overlap between autism and AS and further suggests that dysregulation of UBE3A may play a role in the causation of autism.

Probable new syndrome with Hypomelanosis of Ito-triphalangeal thumb, peculiar facies, cutis gyrata, dental anomalies, and normal intelligence. *E.J. Ramirez-Lizardo^{1,2}, S.E. Totsuka-Sutto¹, J.R. Corona-Rivera^{1,3}.* 1) Laboratorio de Genetica Humana, Universidad de Guadalajara CUCS; 2) Departamento de Genética, IJCR Secretaria de Salud Jalisco; 3) OPD H Civil Juan I Menchaca. Guadalajara, Jalisco México. elizardo@cucs.udg.mx.

Hypomelanosis of Ito (HI) is characterized by unilateral or bilateral macular hypopigmented whorls, streaks, and patches. Abnormalities of the eyes, musculoskeletal, and central nervous systems occur in some cases. Triphalangeal thumb (TT) has been reported in two previous cases of HI. We report a male patient with HI-TT associated to peculiar facies, cutis gyrata on forehead, dental anomalies, and normal intelligence proposed as a probable new mendelian syndrome. The propositus was born at term after an uncomplicated pregnancy and normal vaginal delivery to a 43-year-old G10, P7, A3 mother and 48-year-old father. TT was noticed at birth, and ridged forehead at 2-month-old. Psychomotor development and scholastic achievements were normal. First dentition was referred at age of 6-month-old and permanent dentition at age of 7-year-old. The patient reported a third permanent supernumerary complete ectopic dentition at age of 10-year-old. At 20-year-old showed weight 67 kg (0.2 SD), height 167 (0.8 SD), and OFC 52 cm (-2.1 SD); peculiar facies, striking ridged forehead, synophrys, mild bilateral ptosis, apparent hypertelorism, broad nose, localized hypoplastic amelogenesis imperfecta, 2nd premolar and 1st molar were bilaterally supernumerary on upper archade and were located on the palatal aspect. The skin showed many depigmented areas in linear and whorls patterns with a localized hypertrichosis area over a hypopigmented patch on back. TT on left hand and bilateral pes cavus were also observed. Histologically, ridged forehead was compatible with cutis gyrata. We included differential diagnosis with other syndromes with HI, TT, cutis gyrata and dental anomalies. We failed to find an entity for this pattern of defects and may thus represent new syndrome with skin (HI, cutis gyrata) and skeletal anomalies (dental and thumb anomalies), with possible autosomal dominant inheritance, based on observed advanced paternal age.

A case of neonatal progeria with findings suggestive of a connective tissue disorder. *D.L. Skidmore¹, W. Halliday², S. Viero², V. Edwards², G. Somers², K. Liu³, A. Hinek³, K. Chong^{1,4}, D. Chitayat^{1,4}.* 1) Clinical Genetics and Metabolics, Hospital for Sick Children, Toronto, Ont., Canada; 2) Division of Pathology and Laboratory Medicine, Hospital for Sick Children, Toronto, Canada; 3) Division of Cardiovascular Research, Hospital for Sick Children, Toronto, Canada; 4) Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, Canada.

Neonatal progeroid syndromes are recognisable at birth with a wizened face, wrinkled skin, reduced subcutaneous fat and low birthweight. Subsequent growth is poor and most patients have developmental delay. We report a case of neonatal progeria with a strong similarity to either Megarbane-Loiselet or DeBarsy Syndromes. As has been previously proposed, findings were suggestive of a connective tissue disorder. This male child was born to a consanguineous couple following a pregnancy complicated by oligohydramnios and decreased fetal movement. At birth, he was noted to have a wizened facial appearance, pinched nose and prominent jowls. His entire skin was thin, with prominent vessels and decreased turgor. There were progressive flexion contractures of the fingers, elbows and knees. MRI showed tortuous cerebral vessels and several areas of brain parenchymal haemorrhage, subsequently confirmed on autopsy. The child perished at 6 months of age for unclear reasons. Histological examination of his congenital corneal opacities revealed an epithelium of varied thickness and an absence of Bowman's membrane, as has been previously reported in DeBarsy Syndrome. Immunohistochemistry demonstrated the presence of Collagen types I, II, III, IV. The amount Collagen type I and elastin were decreased on skin biopsy. On electron microscopy, the collagen filaments were normal, however the elastin fibres appeared moth-eaten. Procollagen I and III electrophoresis was normal. In culture, the production of elastin and collagen type I were normal and fibronectin was increased. There appeared to be an intracellular accumulation of procollagen. This case again suggests that some forms of neonatal progeria syndromes fall with the spectrum of connective tissue disorders.

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Concomitant Partial Trisomy 16q and Partial Monosomy 14q: A case report. *L.D. Smith¹, J.E. Sampson¹, S.A. Smith².* 1) Dept Molecular and Medical Genetics, Oregon Health and Sci Univ, Portland, OR; 2) Department of Pediatrics, Oregon Health and Science University, Portland, OR.

Partial trisomy 16q and partial monosomy 14q are rare genetic anomalies with identifiable phenotypes. We present the first patient in the literature to have an unbalanced translocation between chromosomes 14 and 16 resulting in simultaneous occurrence of both of these rare genetic conditions. There is overlap in the reported phenotypes for each of these aneuploidies and our patient exhibited features consistent with both syndromes. Features of trisomy 16q were dominant, manifesting as intestinal and pulmonary vascular abnormalities. Comparing the genotype and phenotype of this patient with previously reported cases provides further refinement of the phenotype-genotype map of 16q proposed by Brisset et. al. (2002).

Growth Assessment of Smith-Magenis Syndrome (SMS). *A.C.M. Smith^{1,2}, A.K. Leonard¹, A. Gropman^{1,2}, D. Krasnewich¹.* 1) MGB/NHGRI/NIH, Bethesda, MD; 2) Georgetown Univ. Med Ctr, Washington, DC.

Smith-Magenis syndrome (SMS) is a well-described, clinically recognizable syndrome associated with an interstitial deletion of chromosome 17p11.2; however, the natural history remains less defined. Over two thirds exhibit short stature, and failure to thrive is frequently seen in infancy. An assessment of anthropometric standards [weight (kg), length/height (cm), head circumference (cm), body mass index (BMI)] was performed on 54 children (22M/32F) with a confirmed deletion diagnosis of SMS and born between 1980-2002. A combination of longitudinal and cross sectional data was collected during evaluation and record review. Data were arranged using mathematical methods to construct gender-based growth curves by age (0-24 months & 2-14 years). Curves were compared to normative data derived from the CDC-National Health & Nutrition Examination Survey (NHANES 2000). BMI was calculated (kg/m^2) in cases where height and weight were simultaneously available. Results: Birth parameters are within the normal range for both sexes. Mean birth length is 49.50cm (50%) for males and 48.77cm (25-50%) for females. Mean birth weight is 3.11 kg (5-10%) for males and 3.05 kg (25%) for females. Mean birth head circumference (HC) is 34.9cm (25%) for males and 33.6cm (25%) for females. Poor growth (5%) is noted within the first 6 months of age and may persist into early childhood. HEIGHT: Males track at 5% through 3 yr, increasing to 25% by 14 yr. Females are at 5% until age 3 yr, then track between 5-25% from 3-7 yr, followed by a decline to <3% by age 12 yr. WEIGHT: Males decline after birth to 5% by age 5 months, then steadily increase to 25% by 6 yr and are at 90% by 14 yr. Females exhibit a brief decline in weight before age 6 months, then track at 25-50% from 1-7 yr with the majority reaching >90% by age 12 yr. HC is normocephalic in the majority. BMI values are variable across ages. This study represents the first systematic assessment of growth in SMS and offers clinicians a means to identify clinically significant growth problems in this rare syndrome.

High cognitive functioning in Pallister-Killian syndrome. *H. Stalker*^{1,2, 3}, *R. Zori*^{1,3}, *B. Gray*^{1,3}, *A. Bent-Williams*^{1,3}, *F. Dolwick*², *C. Williams*^{1,3}. 1) Div Genetics, Dept Pediatrics, Univ Florida, Gainesville, FL; 2) Craniofacial Center, Univ Florida, Gainesville, FL; 3) Raymond C. Philips Unit, Univ Florida, Gainesville, FL.

Pallister-Killian syndrome (PKS) is a rare syndrome of multiple congenital anomalies attributable to the presence of a supernumerary isochromosome 12p. The syndrome presents with characteristic features including: pigmentary skin changes, facial dysmorphia (sparse anterior scalp hair, flattened midface, macrostomia, and coarsening of facial features) and developmental delay. The developmental phenotype of PKS is quite variable; but most are considered to fall into the profound range of developmental functioning. We report on an individual with classical features of PKS with development significantly better than that reported in the literature. As an infant, the patient was hypotonic with sparse anterior scalp hair and distinctive facial features. Chromosome studies performed in childhood revealed an isochromosome 12p in 15 of 52 cells examined in skin fibroblasts, following a normal peripheral blood study. At 14 years of age, she presented to the craniofacial center at the University of Florida for evaluation for midface distraction surgery due to severe midface hypoplasia. FISH studies on buccal mucosa using the D12Z3 probe revealed the presence of the isochromosome 12p in 34 of 150 cells examined. She was noted to have streaky skin hyperpigmentation and has had progressive coarsening of facial features consistent with PKS. Her developmental course has been noteworthy in that a Stanford Binet study at age 4 years revealed a full scale IQ of 83 and at 13 years revealed an IQ of 93. At age 14, the Kaufman Test of Educational Achievement (K-TEA) test also revealed a normal level of functioning with age equivalent scores ranging from 9 years 9 months to 18 years 3 months with relative weaknesses in mathematics and relative strengths in reading and decoding. This child demonstrates a milder developmental course of PKS and highlights the need to consider chromosomal analysis for this condition in individuals with normal intelligence if other physical phenotypic features are present.

ICF syndrome with no mutation in DNMT3B. *M. Suzuki¹, T. Iwai¹, M. Sone¹, K. Miyamoto², H. Yamagata², I. Kondo².* 1) Department of Pediatrics, Kagawa Childrens Hospital, Zentuuji, Kagawa, Japan; 2) Department of Medical Genetics, Ehime University School of Medicine, Ehime, Japan.

The immunodeficiency-centromeric instability-facial anomalies (ICF) syndrome is a rare autosomal recessive disorder characterized by recurrent infections with defective cellular immunity, mild facial dysmorphism and developmental delay. The most diagnostic feature is the pericentromeric instability of chromosomes 1, 9 and 16. An impaired function of DNA methyltransferase 3B (DNMT3B) is causal of this disease. DNA mutations in the DNMT3B gene have been identified in at least 30 patients with the ICF syndrome, but some patients with the ICF syndrome did not have any mutations in the literature, suggesting genetic heterogeneity for the clinical conditions.

We have experienced a case of a female patient with ICF syndrome who suffered of recurrent respiratory infections after three months of age. She was born at term, weighing 1,770g, to no consanguineous healthy parents. When she was hospitalized at 4months of age ; immunodeficiency (IgG 23 mg/dl, IgM 5 mg/dl, IgA 6 mg/dl) was noted. The subsets of T cells and B cells were 86.5% and 5.6%, respectively. She had a typical ICF face and growth and developmental delay. Chromosome analysis showed multiradial configurations involved the heterochromatin regions on chromosomes 1 and 16 in cells examined (16%). She died of Pneumocystis Carinii pneumonia when she was 16months old. DNA analysis were performed in the entire coding and promoter regions of DNMT3B using ABI310 direct sequencer. However, no mutations were detected in DNMT3B.

Clinical features in this patient were identical to most cases with DNMT3B mutations. However, pre- and postnatal growth and development were severely retarded in our case compared to those in patients with DNMT3B mutations. These results suggest that the second gene responsible for the ICF syndrome might have important role in development and growth in addition to immunity.

Teebi-Shaltout Syndrome: A Report of Further Arab Patients. *A.S. Teebi^{1,2}, I. Haroun¹, I. Ahmed³, M.S. Al-Mureikhi⁴, M.S.Z. Salem⁴, G. Al-Thani⁴.* 1) Div'n Clin & Metab Genetics, Hosp Sick Children, Toronto, ON, Canada; 2) University of Toronto, Toronto, ON Canada; 3) Department of Pediatrics, Um-Alqura University, Makkah, Saudi Arabia; 4) Department of Pediatrics, Hamad Medical Corporation, Doha, Qatar.

Teebi-Shaltout syndrome (TSS) was originally described in 1989 in a patient of consanguineous Arab parents from Kuwait. Another Arab family was reported to have 3 affected children. The parents were first cousin of Tunisian origin living in Germany. Manifestations included slow growing hair, hypertelorism, mild ptosis, absent primary teeth, small mouth, mild micrognathia, camptodactyly and caudal appendage. Additional features included blepharophimosis, microphthalmia, and renal anomalies. Inheritance is likely autosomal recessive.

Here we report on 4 patients from 3 consanguineous Arab families with features suggestive of TSS and some previously unrecognized findings thus expanding its spectrum. The first family with a 5-year old male and a 7-year old female sibling from Saudi Arabia. Manifestations included unusual hair, mild hypertelorism, blepharophimosis, small mouth with normal primary teeth, camptodactyly of the 3rd and 4th fingers, and a caudal appendage in one. Intelligence was within normal range.

The other two families are distantly related but from the same large Qatari tribe each with one affected child (a male and a female). Manifestations included a triangular face, blepharophimosis, small mouth with thick lips, long fingers with camptodactyly of 3rd and 4th fingers, sacrococcygeal dimple with a small caudal appendage in one, long big toes, and syndactyly between 2nd and 3rd, and 4th and 5th toes in both feet. Psychomotor development in the 4 patients was within normal.

Based on this report and the previous 2 reports of Arab patients, it appears that TSS is probably not very rare among Arabs and that its pattern of anomalies should be considered when evaluating patients with camptodactyly and/or caudal appendage.

Beckwith-Wiedemann syndrome in a child with subtle phenotype conceived by intracytoplasmic sperm injection: case report and review. *L.N. Teed, A. Ahmad.* Genetics and Metabolism, Children's Hosp of Michigan, Detroit, MI.

Beckwith-Wiedemann syndrome (BWS) is characterized by macrosomia, hemihypertrophy, macroglossia, visceromegaly, omphalocele, ear creases and pits, and an increased risk of embryonal tumors. BWS can result from a number of different genetic mechanisms involving chromosome 11p15: chromosome rearrangements; paternal UPD for chromosome 11; methylation abnormalities of the genes *KCNQ1OT1/LIT1* and *H19*; and mutations in the *CDKN1C* gene. Recent reports have noted an increased frequency of conception via in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) in children with BWS.

We report a case of BWS in a girl conceived by ICSI, who presented for evaluation at 2 years 9 months of age. The pregnancy was uncomplicated with a normal ultrasound examination. She was born at 35 weeks gestation by spontaneous vaginal delivery. Birth weight and birth length were both greater than the 90th percentile for gestational age. There was no known history of neonatal hypoglycemia. Macroglossia and an umbilical hernia were noted in the newborn period but a diagnosis of Beckwith-Weidemann syndrome was not considered. At presentation to genetics clinic at 2 years and 9 months, her height was 97 cm (90th percentile) and weight was 15 kg (75-90th percentile). On physical examination, she had mild macroglossia with a tendency to hold her mouth open and a small umbilical hernia. The physical exam was otherwise completely normal with no ear creases or pits, hemihypertrophy, or visceromegaly. Family history was unremarkable. Review of photographs confirmed the macroglossia was more apparent at younger ages. Peripheral blood karyotype was normal. Molecular analysis detected hypomethylation of the *KCNQ1OT1/LIT1* gene.

This report adds to the previously reported cases of BWS in the medical literature (about 20 total cases) associating assisted reproductive technologies with epigenetic alterations. We suggest that conception via IVF or ICSI be considered a supportive finding in children with features, albeit subtle, of BWS.

Familial Sotos syndrome with unusual clinical manifestations. *D.H. Tegay, J. Weiss-Burns, P. Galvin-Parton.* Stony Brook University Hospital, Stony Brook, NY.

Sotos syndrome is characterized by childhood overgrowth, advanced bone age, macrocephaly, distinctive facial features and developmental delay. Mutations or deletions involving NSD1 are causative and follow autosomal dominant inheritance. Frequently reported associated features include hypotonia, congenital heart disease, seizures, scoliosis, strabismus, and cancer. Rare familial cases have been reported and decreased fertility has been implied.

Here we report a case of a 5 year-old Caucasian female with typical features of Sotos syndrome ascertained during evaluation for severe glomerulonephritis secondary to systemic lupus erythematosus. NSD1 DNA sequence analysis revealed a novel single base pair deletion predicted to be pathologic for Sotos syndrome (6291delG) due to the frameshift creation of a downstream premature stop codon. Due to large stature, learning disability and distinctive facial features, testing was carried out on the 44 year-old mother of the proband and also revealed the causative 6291delG NSD1 mutation, confirming familial Sotos syndrome.

NSD1 is expressed in a variety of tissues including brain, kidney, muscle, spleen, thymus and lung, where it appears to be involved in chromatin regulation and interacts with a variety of nuclear receptors. Systemic lupus erythematosus has not previously been reported in association with Sotos syndrome. The severity of this patient's lupus glomerulonephritis (WHO class IV) together with a prior case report describing increased severity of familial ADPKD in an individual with concomitant Sotos syndrome suggests the possibility of a deleterious effect of NSD1 mutations on the kidneys.

Tricho-Hepato-Enteric Syndrome: a report of 2 further cases with a novel pattern of iron accumulation and abnormal platelets. *R.E. Teitelbaum¹, D.L. Skidmore^{1,4}, R. Moodie³, W. Kahr⁵, N. Martin¹, J. Kingdom², N. Okun², Y. Diambomba^{3,6}, S. Viero⁵, S. Unger⁴, N. Querica⁴, D. Chitayat^{1,4}.* 1) Prenatal Diagnosis & Medical Genetics, Mount Sinai Hospital, Toronto, ON, Canada; 2) The Department of Obstetrics and Gynecology, MSH, Toronto, Canada; 3) Department of Neonatology, Hospital for Sick Children, Toronto, Canada; 4) Clinical and Metabolic Genetics, HSC, Toronto, Canada; 5) Department of Pediatric Laboratory Medicine, HSC, Toronto, Canada; 6) The Department of Neonatology, MSH, Toronto, Canada.

Tricho-Hepato-Enteric syndrome (THES) is a disorder which consists of: abnormal hair, liver dysfunction, abnormal hepatic iron storage and intractable diarrhea. The condition was first reported by Stankler et al(1982) and further delineated by Verloes et al(1997). We report sisters affected with this condition and further delineate the clinical manifestations. The first child died at 6 month of age due to sepsis, intractable diarrhea and liver dysfunction. She was noted to have FTT, congenital hyperbilirubinemia, thrombocytopenia, possible immunodeficiency, VSD, mild facial dysmorphism, "woolly" hair and liver fibrosis. Histology showed iron deposition in the Kupffer Cells of the liver and at the apical end of the thyroid follicles. Bowel biopsies were non- diagnostic. Platelet microscopy revealed giant platelets with canalicular dilatation, vacuolation and abnormal content. Her karyotype was 46,XX. The second pregnancy was complicated by mild IUGR and transiently distended bowel loops at a gestational age of 30 weeks. Postnatal examination showed a female child with woolly hair and similar dysmorphisms. At two weeks of life, she developed intractable diarrhea. An MRI inferred the presence of metal in the liver and thyroid, but not the pancreas, spleen or heart. Like her sibling, her ferritin was elevated and her platelets had similar abnormalities. Furthermore, some of the platelets had an almost complete absence of microtubules. It appears that in addition to previously reported findings that THES has a distinct pattern of iron deposition and novel hematological findings.

Rare case of median cleft of the upper lip with pedunculated skin-covered mass presented through nostril. M.

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A median cleft of the upper lip occurs either as an isolated anomaly or as a part of well-recognized syndromes (Majewski, OFD, Ellis-van Creveld, holoprosencephaly, etc). In a frontonasal dysplasia sequence, a midline cleft of the upper lip may continue through the nose (a bifid nose) up to widely spaced eyes (hypertelorism). However, only approximately 25 authors have described patients with a median cleft of the upper lip, double frenum, hamartomas, and a nasal mass extending from columella. Our patient was a 6 months old baby girl who presented with a slight facial asymmetry, midline defect of the upper lip with a small midline defect of the alveolar maxillary ridge, and a pedunculated skin-covered club-shaped cylindrical mass protruding through a left nostril. The face was asymmetrical with a wide nasal bridge. The left palpebral fissure and the left eye were smaller. The girl was born as a second child to a 20 years old mother and to a 36 years old father. Mother had a first child from her previous marriage and father had three healthy children from his previous marriage. Birth weight was 6 lbs 2 oz. The mother had a cold in the third month of pregnancy. The younger brother of the mother was born with cleft lip and palate. A CT scan revealed that there is no communication with nasal or frontonasal sinuses. It also excluded a frontonasal encephalocele. The girl underwent surgery under general anesthesia. A pedunculated mass that was attached to the nasal septum was successfully removed. The nasal airway was clear after excision of the nasal mass and the girl could easily breathe through her nose. As the mass involved a margin of the left ala nasi, a defect of the left nostril occurred after the excision. It could be corrected later. Histological examination showed that the mass consisted of skin, fibrous connective tissue, and adipose tissue. The child was released from the hospital the following morning and recovered successfully. The study of this case, as well as the surgery, was supported by the Rotaplast International, Inc.

A New Syndrome of Congenital Extraocular Muscle Fibrosis and Ulnar Hand Anomalies Maps To Chromosome

21qter. *T. Tükel^{1,4,5}, A. Uzumcu⁵, A. Gezer⁶, H. Kayserili⁵, M. Yuksel-Apak⁵, O. Uyguner⁵, S.H. Gultekin³, H.C. Hennies⁷, P. Nurnberg^{7,8}, R.J. Desnick^{1,2}, B. Wollnik⁵.* 1) Department of Human Genetics, Mount Sinai School of Medicine, New York, NY; 2) Department of Pediatrics, Mount Sinai School of Medicine, New York, NY; 3) Department of Pathology, Mount Sinai School of Medicine, New York, NY; 4) Department of Genetics, Institute for Experimental Medicine (DETAE), Istanbul University, Istanbul, Turkey; 5) Division of Medical Genetics, Child Health Institute, Istanbul University, Istanbul, Turkey; 6) Department of Ophthalmology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey; 7) Gene Mapping Center and Department of Molecular Genetics, Max Delbrück Center for Molecular Medicine, Berlin, Germany; 8) Institute of Medical Genetics, Charité, Humboldt University Berlin, Berlin, Germany.

We report a new autosomal recessive syndrome of congenital fibrosis of extraocular muscles (CFEOM) with notable ulnar hand abnormalities. All six affected patients from a large consanguineous Turkish family, aged 2 to 29, had 1) nonprogressive restrictive ophthalmoplegia with blepharoptosis primarily of the right eye and 2) postaxial oligodactyly/oligosyndactyly of varying severity in both hands, but always more severe on the right. A genome scan at a marker density of about 7 cM established linkage of the disease locus to chromosome 21qter. The multipoint LOD score was 4.53 at 55cM on chromosome 21 and recombinations defined the critical region as ~1.5 cM between microsatellite D21S1897 and the telomere of the long arm. This region contains about 15 genes and 10 open reading frames but no obvious candidate genes. Clinical findings of the patients and fine mapping of the locus are presented in detail. Identification and functional studies of the syndrome-causing gene may provide insights into the development of the extraocular muscles and the cranial motor nuclei, as well as anteroposterior limb development.

Cytogenetic and clinical description of the 9p deletion syndrome. *H. Vance¹, C. Crowe², L. Christ¹, M. Eichenmiller¹, M. Graf¹, A. Matthews¹, T. Hassold¹, S. Schwartz¹.* 1) Case Western Reserve Univ, Cleveland, OH; 2) MetroHealth Med Ctr, Cleveland, OH.

The 9p deletion syndrome (9p-) is characterized by trigonocephaly, typical facial features, long middle phalanges, umbilical and inguinal hernias, abnormal genitalia, cardiac abnormalities, and hypotonia. All patients have some degree of cognitive impairment. The critical region for 9p- has been localized to a region between 9p22.3-p23. To better define the cytogenetic and clinical manifestations resulting from a deletion of the critical region, 35 patients were identified with cytologically terminal deletions of chromosome 9p proximal to this critical region. High-resolution chromosome analysis, microsatellite analysis, and BAC analysis were utilized to define breakpoints, deletion sizes, and parental origins of the deletions. Clinical data on each patient was obtained by physical examination and review of medical history. Cytogenetic and molecular analysis revealed that: 1) breakpoints are heterogeneous, with deletions in this group ranging in size from 14.1 to 19.6 Mb; and 2) paternally derived deletions occur significantly more frequently than maternally derived deletions. Clinical data analysis demonstrated that: 1) all patients with deletions proximal to the 9p-critical region have phenotypic manifestations characteristic of 9p-; 2) there is great variability in the frequency and severity of phenotypic expression; 3) several trends suggestive of karyotype/phenotype correlations were observed; and 4) certain findings were noted only in specific subgroups of patients with larger deletions, including tapered fingers, sandal toe gap, nystagmus, and rockerbottom feet, suggesting more proximal localization of these traits. These observed trends may partially contribute to the clinical variability of 9p-. However, results from this study indicate that breakpoint analysis alone cannot predict phenotype. Thus, following the prenatal or postnatal identification of a deletion of the 9p critical region, genetic professionals should continue to emphasize the clinical variability of 9p- to parents and families and provide a thorough evaluation and appropriate management.

The mute Elfin : Williams syndrome and autism. Report of 5 patients. A. Verloes¹, P. Blanchet², A. Toutain³, R. Touraine⁴, D. Héron⁵, O. Dupuy¹, C. Baumann¹, P. Saugier-veber⁶, E. Excoffier⁷, D. Cohen⁸, S. Tordjman⁹. 1) Clinical Genetics Depts, Robert Debré Hosp, Paris; 2) Montpellier University Hosp; 3) Tours University Hosp; 4) Saint-Etienne University Hosp; 5) La Pitié-Salpêtrière Hosp, Paris; 6) Rouen University Hosp; 7) Child Psychiatry Depts, Robert Debré Hosp, Paris; 8) La Pitié Salpêtrière Hosp, Paris; 9) Rennes University Hosp, France.

Williams syndrome (WS) is a rare genetic condition whose clinical manifestations include a distinct facial appearance, CHD, neonatal hypercalcemia, and a cognitive and behavioral phenotype characterized by a relative preservation of linguistic and social-affective skills ("cocktail party" personality with expansive and expressive speech) and musical ability in the face of profound impairment of visuo-spatial abilities and numeric processing. Domains of socialization and communication are considered to be relative strengths, whereas daily living and motor skills are relative weaknesses in WS. We report 5 WS patients with MR and pervasive developmental disorder (PDD) of the autistic type (according to DSM IV criteria) resulting in e.a. in total absence of language. Evaluations of the 5 patients by ADI and CARS confirmed the clinical diagnosis of PDD. All patients were deleted by FISH in 7q11.23. At time of submission, investigation by QMPSF for 9 exonic amplicons within 7 different genes inside the common 1.5 deletion and 2 genes at the boundaries confirmed the presence of the common deletion in 2/5. Only 2 previous reports mention coexistence of WS and PDD in the literature. Causal relationship between WS and PDD will be discussed. Despite verbal fluency, it appears that WS patients exhibit PDD-related behaviors, such as hyperacusis, obsessive interests, global spatial perception deficit, Asperger-like verbal prosody, inflexibility, ritualism, obsessiveness, and perseverating attitudes. More severe PDD behavior may be more common in WS than reflected by literature scan. A possible hypothesis would be a mutation in the second allele of a gene involved in neuropsychological functions in WS genomic interval leading to total inactivation of the gene expression.

Obesity and Stature in Adults with 22q11 Deletion Syndrome. *V.W.Y. Wong^{1, 2}, E.W.C. Chow^{1, 2}, O. Caluseriu^{1, 3}, R. Weksberg^{2, 3}, A.S. Bassett^{1, 2}.* 1) Clinical Genetics Research Program, Centre for Addiction and Mental Health (CAMH), Toronto, ON, Canada; 2) University of Toronto, Toronto, ON, Canada.; 3) Hospital for Sick Children, Toronto, ON, Canada.

Background: 22q11 Deletion Syndrome (22qDS) is a common microdeletion syndrome. Up to 30% of adults may develop schizophrenia (SZ). While children with 22qDS have been reported to have short stature and failure to thrive, there is limited information on adult parameters. Method: Height (Ht) and Weight (Wt) of 72 adults (age >17 y) with 22qDS confirmed using standard FISH methods (age > 17) were measured. There were 38 female and 34 male subjects assessed at mean age 28.8 y (SD = 10.1 y). 35 subjects (48.6%) had psychotic illnesses treated with medications that can cause weight gain; 16 subjects had a history of hypothyroidism. Adult body mass index (BMI), Ht and Wt percentiles were calculated using US Centre for Disease Control Growth Charts, which account for age and sex. Results: There is a significant difference in Ht between Male and Female as expected ($p = <0.0001$) and Wt ($p = 0.0028$), but not in BMI ($p = 0.9597$). There was no significant difference on any measure between subjects who had or did not have a psychotic illness, or those with or without hypothyroidism. Comparing the 22qDS sample with general population (age and sex corrected) percentile norms, significantly greater proportions of subjects with 22qDS had short stature: height <3%ile ($n = 13$; 18.31%; $\chi^2 = 11.47$; $df = 1$; $p = 0.0007$), <10%ile ($n = 30$; 42.25%; $\chi^2 = 24.10$; $df = 1$; $p = <0.0001$), and obesity: BMI >90%ile ($n = 31$; 43.66%; $\chi^2 = 25.81$; $df = 1$; $p = <0.0001$). BMI ranged from 20.4 - 38.1 for men and 17.2 - 48.3 for women. Only 4 subjects (5.6 %) were underweight. Conclusions: Overall, adults with 22qDS are shorter in stature and have more obesity than the general population. Weight gain in 22qDS is not primarily associated with anti-psychotic medications or thyroid disease, and represents a major health issue for these patients.

Schizophrenia as the presenting feature of Velo-cardio-facial-syndrome: effect of targeted therapy. *H. Yonath, C.W. Brown.* Dept Molecular & Human Gen, Baylor College of Medicine, Houston, TX.

The clinical presentation of the 22q11.2 deletion syndrome (22q11.2 DS) can be quite variable, and the characteristic features may include congenital heart disease, palatal abnormalities, immune deficiency, facial dysmorphism, learning disabilities, and psychiatric illness. Hypocalcemia, feeding difficulties, renal anomalies, and hearing loss are also common. Although patients with 22q11.2 DS are usually diagnosed during early childhood, we report a case in which the syndrome was diagnosed in an 18-year old female referred to our Genetics clinic due to acute psychosis that had responded poorly to medical therapy. Physical examination revealed a long face, bulbous nose, prominent upper lip, protuberant asymmetric ears, nasal voice, webbed neck, and tapering fingers. Cardiac exam and stature were normal. Echocardiogram and renal sonogram showed no structural abnormalities. Fluorescence in situ hybridization identified a characteristic 3 Mb 22q11.2 deletion. The gene encoding catechol-o-methyltransferase, an important enzyme for dopamine metabolism, lies within the deletion interval and may be relevant to the formulation of a more targeted treatment plan for these patients. Some 22q11.2 DS patients who are refractory to conventional forms of therapy for schizophrenia improve when treated with Metyrosine, an inhibitor of tyrosine hydroxylase. Although our patient showed a good initial response to Metyrosine, her psychiatric status worsened within a few weeks as the dose of the drug was increased. L--methyldopa, which inhibits the decarboxylation of dopa to dopamine, is currently being considered. This patient represents an example of the wide variability of 22q11.2 DS, and manifests the need for increased awareness of this disorder when evaluating schizophrenic patients.

Another Ptosis Loci? Distal 3q beyond BPES. *D. Zand, K. Rosenbaum.* Dept Genetics and Metabolism, CNMC, Washington, DC.

With the recent commercial utilization of fluorescence in situ hybridization (FISH) technology, diagnoses of cryptic subtelomeric deletions have been more common. Some subtelomeric deletions are reported frequently, and good description is available within the literature (J Med Genet 2003:40:385-398). The chromosome 1p subtelomeric deletion has become to have a recognizable phenotype. Children with other deletions have not yet been as well delineated. For example, subtelomeric deletion of 3q is much less common, with only one report (J Med Genet 2001:38:417-20) in the literature. That report noted facial dysmorphism and moderate mental retardation in a two-year old child, but no further elaboration was available. We present a nine-year old pre-pubertal Caucasian male with a de novo subtelomeric deletion on chromosome 3q. He presented with a history of multiple surgeries to correct bilateral ptosis, and mild developmental delay accompanied by behavioral outbursts. On physical exam his growth parameters were on the growth chart, but he had very mild dysmorphism which included ptosis. He had an elevated palate, simple ears, malar flattening, and camptodactyly of his DIP joints. Additionally he had diffuse eczema and a monotone, hoarse voice. By comparison, a two-year old patient with an interstitial deletion of 3q28-3q29, and the subtelomere FISH for 3q present did not have ptosis or other eye findings on clinical exam. Ptosis is a relatively non-specific dysmorphism, and has been associated with many syndromes. FOXL2, the gene mutated in blepharophimosis, ptosis, and epicanthus inversus syndrome (BPES, OMIM 110100) is located on chromosome 3q23, proximal to the subtelomere. There are also reports of ptosis in ring chromosome 3 (Am J Med Genet 39:2:130-2), and distal deletions on chromosome 3p. The paucity of reports of patients with subtelomere 3q deletions raises the possibility for different loci important in eyelid development. Additionally there may be a cis-acting element within the region deleted that may interact with other known loci, such as FOXL2. Comparison of additional patients, and molecular evaluation of their individual deletions will be essential.

A contiguous gene syndrome involving both Elastin gene and ABCB4 gene due to interstitial 7q11.22-q21.2

deletion. *M. Aminah*¹, *Y.S. Choy*², *S.K. Tan*¹, *A. Ruziana*³, *C.B. Lim*³, *N.L. Lim*⁴. 1) Cytogenetic Lab. , Kuala Lumpur Hospital, Kuala Lumpur, Malaysia; 2) Genetics & Metabolism Unit, Pediatric Institute, Kuala Lumpur Hospital, Malaysia; 3) Hepatology Unit, Pediatric Institute, Kuala Lumpur Hospital, Malaysia; 4) Pediatric Department, Selayang Hospital, Malaysia.

The loss of a segment of adjacent/contiguous genes due to the deletion of a segment of chromosome is an important cause to explain an array of apparently clinically unrelated problems in a patient. We reported here a patient who had a normal routine karyotype reported earlier in another center referred to us for further evaluation of dysmorphism associated with chronic cholestatic hepatitis and multiple problems including hypothyroidism, failure to thrive, multiple birth defects, pulmonary stenosis and mitral insufficiency, cutaneous hyperelasticity and hypermobility of joints as well as bladder diverticulum. In addition, he had mild hypercalcemia, hypertension and developmental delay. Biochemical and pathological assessment confirmed intrahepatic cholestasis without biliary atresia. His facies was typical to that of William syndrome. High resolution karyotyping revealed an interstitial deletion of one of the 7q11.22 to q21.1 segment. This deletion encompassed the Elastin gene responsible for William syndrome at 7q11.23 and ABCB4 gene causing Familial Intrahepatic Cholestasis Syndrome explaining the myriad of clinical signs and symptoms in this patient. Other genes potentially involved in this deletion due to haploinsufficiency include CCM1 gene which causes cerebral cavernous malformation, MHS3 gene that predisposes to malignant hypertension and PTPN12 gene that predisposes to colonic cancer later in life. Detection of the deletion provided invaluable information in counseling and management of the patient.

Chromosome 19 q12.q13.3 deletion is associated with severe developmental delay, autism, and anesthesia and sedative sensitivity. *P. Benke, M. Rodriguez, V. Karamanian, M. Floyd.* Div. Genetics, Dept. Pediatrics, Univ. Miami Sch. Medicine, Miami, FL.

Only one patient with a 19 q12.q13.3 deletion has been reported to date. We report here the subsequent course of this patient, interesting because of the progression of her CNS behaviors and delay to an autism-like state, and important because of her severe sensitivity to anesthetic agents and sedatives. The patient is a 9.5 year old girl with chromosome 19 q12.q13.3 deletion previously described as dysmorphic, SGA, and delayed (Kulharya et al; *Amer. J. Med. Genet.* 77:391, 1998). Dysmorphic features, including small head, small size, minor facial anomalies (low set ears, epicanthic folds), oral dysphagia, deafness, brain atrophy, heart anomalies, kidney anomalies and severe developmental delays were observed. She progressed to autistic-like behaviors as she became older, with poor eye focus and interactions, repetitive utterances and hand motions/hand flapping, and self-mutilation and self-stimulation activities. She could stand but not walk, she did not talk, she had poor sleep, and she understood very little. She indicated hunger with increased irritability. Severe drug-sedative sensitivity was observed when she received 30 mg. chloral hydrate for a CNS scan, and then required resuscitation/intubation followed by emergency intervention for a cardiopulmonary arrest 6 hours later. Chronic reflux and poor weight gain led to a fundoplication and G-tube placement. Prolonged recovery time from anesthesia/ketamine and a second arrest followed this procedure. Enzymes that metabolize drugs, including cytochrome oxidase and p450 enzymes are located on chromosome 19q13.1-13.2, and it is likely that their function was compromised by the chromosome deletion. Deletion mapping using microsatellite markers revealed that the deleted region was between Markers DS 19S425 and DS19S412, which contains genes for P450 and cytochrome oxidase. A 19q locus for autism has been suggested. Chromosome 19 q12.q13.3 deletion is a severe MR-dysmorphic-autism-like disorder and is associated with increased anesthesia and sedative drug sensitivity.

Survival to age 40 months in a 69,XXY infant. *R. Best¹, L. D'Souza¹, K. Brooks¹, K. Corning², K. Clarkson².* 1) Dept Obstetrics/Gynecology, Univ South Carolina Sch Med, Columbia, SC; 2) Greenwood Genetic Center, Columbia, SC.

Triploidy, the presence of a complete extra set of chromosomes, is a chromosomal abnormality where the conceptus receives a double chromosomal contribution from one parent. Triploidy accounts for 6% of spontaneous abortions, while only 1% of triploid conceptions survive to term. Triploid fetuses can be classified either as a Type I triploid of paternal (diandric) origin with mild to moderate intrauterine growth retardation (IUGR) and a partial hydatidiform mole, or as a Type II triploid of maternal (digynic) origin having severe, asymmetrical IUGR. Survival of live born triploids is higher for those of digynic origin, and thus far no triploid has been reported to survive beyond 312 days. We report here a triploid male who survived 40 months. The infant was born to a 29-year old healthy female at 30 weeks gestation and required NICU admission for respiratory distress. At birth, the infant weighed 1102 g and presented with several physical abnormalities. Physical examination showed bitemporal narrowing, flat nasal bridge, small mouth, wide alveolar ridge, narrow high palate, patent ductus arteriosus, primary hypospadias, bilateral scrotal hydroceles, hypoplastic nipples, bilateral inguinal hernias, bilateral transverse palmar creases and bilateral syndactyly of the toes. Congenital optic nerve atrophy, macular scarring, and absence of the gallbladder were noted. Lab studies were initiated to exclude Smith-Lemli-Opitz syndrome and cytogenetic abnormalities. 17-dihydrocholesterol values were within normal limits. Cytogenetic analysis of 50 metaphase cells from mitogen-stimulated peripheral blood cultures revealed a 69, XXY karyotype without evidence of mosaicism. The family declined study of skin fibroblasts for further study of mosaicism as well as molecular studies to confirm parental origin. Clinical findings and the extraordinary survival of this child are suggestive of digynic origin.

Hypomelanosis of Ito associated with de novo Extra Structurally Abnormal Chromosomes. *Y.S Choy¹, A. Ruziana², S.K. Tan², L.H. Ngu¹, W.T. Keng¹, I.H.M. Hussain¹.* 1) Genetics & Metabolism Unit, Pediatric Institute, Kuala Lumpur Hospital, Kuala Lumpur, Malaysia; 2) Cytogenetic Laboratory, Kuala Lumpur Hospital, Malaysia.

Hypomelanosis of Ito is a neurocutaneous phenotype with skin hypopigmentation along the lines of Blascko associated with multiple extracutaneous manifestations, mostly of central nervous and musculoskeletal origin. The pathogenic etiologies are heterogeneous but chromosomal mosaicism has been found in about 50% of individuals. Juxtaposition of normal and abnormal neural cells in the brain parallels the cutaneous findings. Here we report 3 patients with hypomelanosis of Ito having mental, psychomotor and speech retardation, hypotonia and behavioral problems associated with de novo extra structurally abnormal chromosomes (ESAC). One of them has a karyotype of 47, XX, +r(X) detected uniformly in all the amniocytes and all the lymphocytes tested. She also has severe bilateral vesicoureteric reflux, dysgenetic corpus callosum and coarse facies. Functional mosaicism of Xp disomy due to the ring lacking in the XIST locus is probably the cause of the disorder as demonstrated in previous studies. The other 2 unrelated patients, a boy and a girl, have an extra bisatellited isodicentric 15 marker chromosome in all the lymphocytes tested. FISH using SNRPN probes confirmed trisomic or tetrasomic dosage of the segment. Apart from the neurobehavioral and cutaneous abnormalities both of them have dysmorphic facies and short stature. Their phenotype is similar to another patient with 46, XY, r(15). Unlike the latter whose hypomelanosis probably due to haploinsufficiency of P genes depending on the size of the ring in different cells, the exact mechanism of hypomelanosis in these two patients is yet to be elucidated.

Program Nr: 718 from the 2004 ASHG Annual Meeting

Chromosome 6 In The Genetic Clinic. *J. Claus, B.G. Kousseff.* Dept Pediatrics, Genetics Div, USF Regional Genetics Program, Tampa, FL.

Between 1/2/81 and 12/31/02 through busy (2002 patient volume 2,797) pediatric and prenatal clinics at the University of South Florida, 42,795 probands/families were evaluated. Thirty-two had anomalous chromosome 6. There were fifteen translocations; t(1;6) was encountered three times, all with different breakpoints. One of them had FISH positive test for DiGeorge syndrome and t(1;6) in his sister. t(3;6) and t(6;9) were encountered twice each, all with different breakpoints, and t(4;6), t(5;6), t(6;7), t(6;12), t(6;14), t(6;15) and t(6;18) were evaluated as singular proband/families each. t(6;6) was a mosaic. Five were maternal, 2 paternal, 3 de novo, 2 had family history of affected individuals and 3 were without complete parental studies. Two probands had complex chromosome rearrangements (CCR) with 4 and 8 breakpoints respectively. The six inversions were 4 pericentric, 1 each maternal, paternal, de novo and 1 with incomplete parental study. The latter had familial Hay-Wells ectodermal dysplasia as well. The two paracentrics were 1 de novo and 1 maternal with X/XX/XXX mosaicism? There were 2 del6q, both de novo (AM J Med Genet 62:105). One dup6q was de novo. There were 4 del6p: 2 de novo and 2 with incomplete parental studies. Among them, 1 de novo and 1 with incomplete study had the same breakpoint, 6p23. Trisomy 6 was encountered in 2 unrelated products of conception as a part of family studies. One of them had a mother with t(10;16), trisomy 16 and triploidy in additional miscarried fetuses. All together 17 proband/families were evaluated through the pediatric clinic, 10 as prenatal patients and 5 as having family history of anomalous chromosome 6. Twenty-five were Caucasians, 3 Hispanic, 1 Afro-American and 3 were biracial. The study emphasized that karyotype/phenotype correlations will be meaningful only if based on large number of patients with completed parental studies. This requires clinical and cytogenetic expertise within the university based genetic programs.

Unsuspected Klinefelter Syndrome diagnosed during oncologic evaluation: a case series. *M.M. Eberl^{1, 2}, M.R. Baer³, L. Fitzpatrick⁴, M.C. Mahoney^{1, 2, 5}, S.N.J. Sait⁶, S. Kakati⁶, A.W. Block⁶, C.D. Farrell⁷.* 1) Division of Cancer Prevention and Population Sciences, Roswell Park Cancer Institute (RPCI), Buffalo, NY; 2) Department of Social and Preventive Medicine, School of Public Health, SUNY Buffalo, Buffalo, NY; 3) Leukemia Section, Department of Medicine, RPCI, Buffalo, NY; 4) Department of Pediatrics, RPCI, Buffalo, NY; 5) Department of Family Medicine, SUNY Buffalo, Buffalo, NY; 6) Clinical Cytogenetics Laboratory, Department of Pathology and Laboratory Medicine, RPCI, Buffalo, NY; 7) Department of Cancer Genetics, Clinical Genetics Service, RPCI, Buffalo, NY.

Klinefelter Syndrome (KS) has significant implications for health and for medical management, however, it remains widely underdiagnosed. Contributing to this lack of recognition is the variable phenotypic expression of KS and the tendency of primary care clinicians to underestimate the prevalence of KS in their patient populations. It is not unusual for KS to elude diagnosis until problems with infertility arise or karyotyping is performed for other reasons, such as the work-up of a malignancy. This report presents one pediatric and five adult male patients with previously undiagnosed KS ascertained as a result of cytogenetic testing for suspected hematologic malignancies. The presenting medical problem, as well as the karyotypic and phenotypic presentations in these cases, will be discussed. Clinical features span the spectrum of classic to subtle phenotypic findings, with age at diagnosis ranging from 7 years old in the pediatric case to 48-67 years in the adult cases. The medical, genetic and psychosocial implications of the KS diagnosis will be explored. Screening and prevention considerations, such as bone density testing in the untreated adult males and timely testosterone supplementation in pediatric cases, will be outlined. Family physicians must become familiar with the more common genetic conditions and their varied presentations. This case series highlights the importance of maintaining a comprehensive and holistic approach to medical care, including regular communications with, and education of, community practitioners.

A locus for Nasal Glioma on chromosome on Chromosome 7p21.1. *S. Ennis*^{1,2}, *K. Ryan*², *P. Carthy*², *A. Dunlop*², *K. Dunne*³, *A.J. Green*^{1,2}. 1) Department of Medical Genetics / Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Crumlin, Dublin 12, Ireland; 2) National Centre for Medical Genetics, Our Ladys Hospital for Sick Children, Crumlin, Dublin 12, Ireland; 3) Dept. of Paediatrics, University College Hospital, Galway, Ireland.

A 2 year old girl was seen in the genetics clinic. She was the second child of healthy unrelated parents, and was referred because of a de novo balanced reciprocal chromosome translocation 46,XX, t(3;7)(p13;p21). Karyotyping had been carried out because of concern over dysmorphic features. The patient was developmentally appropriate, and did not in fact show any dysmorphic features. She had a 1 cm raised lesion on the bridge of her nose, which had been present from birth. MRI scan of her brain did not show any connection between this lesion and the intracranial contents. The lesion was removed, and showed on histology to contain heterotopic glial and neuronal tissue, giving a diagnosis of a nasal glioma. Nasal gliomas are rare usually isolated developmental anomalies, whereby neuronal and glial tissue develops on the bridge of the nose, in the absence of a frontal encephalocele. This individual had a cytogenetically visible de novo apparently balanced reciprocal translocation 46,XX, t(3;7)(p13-p12.3;p21.1). Fine mapping of the 3p and 7p regions was carried out using FISH mapped BACs to establish the location of the break points. FISH analysis indicated a break point between the chromosome 3p clones RP11-90H15 and RP11-79O5 and within the chromosome 7p clone RP11-123E5. Analysis of the break point surrounding regions identified AGR 2 (anterior gradient 2 homolog) a *Xenopus* gene (XAG2), which is critically involved in forebrain development and the most dorsally expressed gene in frog brain development, in close proximity and distal to the 7p break point as a possible candidate gene. A second patient with nasal glioma which occurred as part of a rare genetic disorder, Knobloch syndrome was identified. Sequence analysis of the AGR 2 gene revealed no mutations. We are now recruiting a larger series of patients with non-syndromic nasal gliomas, to look for alterations in the AGR2 gene.

Program Nr: 721 from the 2004 ASHG Annual Meeting

Chromosome 8 In The Genetic Clinic. *G. Jervis*¹, *B.G. Kousseff*², *University of South Florida, Regional Genetics Program.* 1) Clinical, Genzyme Genetics, Tampa, FL; 2) University of South Florida, Regional Genetics Program, Tampa FL.

Between 1/2/81 and 12/31/02 through busy (2002 patient volume 2,797) pediatric and prenatal clinics at the University of South Florida, 42,795 probands/families were evaluated. Forty-nine had anomalous chromosome 8. There were sixteen translocations; t(8;13) was encountered three times, all with different breakpoints. Two had t(5;8) and one each t(1;8), t(2;8), t(3;8), t(4;8), t(6;8), t(7;8), t(8;15), t(8;17), t(8;18), t(8;22) and t(X;8). Six were maternal, 2 paternal, 6 de novo, 1 was with a family history and another with incomplete parental study. One had de novo CCR with 3 breakpoints. There were 11 trisomies, 10 mosaics and 1 trisomy in a POC. The 4 inversions were 2 maternal, 1 paternal and 1 de novo. There were 10 dup 8p; 1 maternal, 6 de novo, and 3 with incomplete parental studies. One of these 3 also had inversion. The 4 del 4p were 2 de novo and 2 with incomplete parental studies. The 3 dup8q were 1 with incomplete parental study and 2 de novo. One of these had also inversion. Thirty probands/families were pediatric, 16 were prenatal, and 3 had family history of anomalous chromosome 8. Thirty-five were Caucasians, 7 Hispanic, 6 Afro-American and 1 was biracial. The study emphasized that karyotype/phenotype correlations will be meaningful only if based on large number of patients with completed parental studies. This requires clinical and cytogenetic expertise within the university based genetic programs.

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CHROMOSOME 5 IN THE GENETIC CLINIC. *M. Jodah, B.G. Kousseff.* Dept Pediatrics, USF Regional Genetics Program, Tampa, FL.

Between 1/2/81 and 12/31/02 through busy (2002 patient volume 2,797) prenatal and pediatric genetics clinics at the University of South Florida 42,795 probands/families were evaluated. Fifty-two had anomalous chromosome 5. Twenty three had translocations; 4 had t(5;7) with different breakpoints. Three each had t(3;5) and t(5;19) respectively. Two each showed t(5;9), t(5;18), t(1;5), t(5;8) and t(2;5) respectively; all with different breakpoints. One each had t(4;5), t(5;6), and t(5;15), respectively. Six were maternal with one showing 47,XXY as well. Three were paternal and 5 denovo. Among the latter there was one with Trisomy 21. Five had complex chromosome rearrangement (CCR) all denovo. Two had 3 breaks each and one each had 6, 8, and 10 breaks respectively. There were 18 probands with del 5p; 9 were denovo with 1 of them having a father with 46,XY/47,XXY. A male with del 5p had a mosaic mother and a female had a mother who also had del 5p. A family was studied for del 5p in a first cousin. A prenatal study showed mosaic del 5p in chorionic villi sampling and 46, XX in amniocytes, pseudomosaicism. The remaining 5 had incomplete parental studies. The 4 inversions were 1 paracentric and 3 pericentric. Two of the 3 pericentric were maternal. The 2 del 5q were interstitial in one and a terminal in another, both denovo. Seventeen were prenatal, 1 had family history of del 5p and 34 were evaluated through the pediatric clinic. Thirty-eight were Caucasians, 8 Hispanic, 5 African-Americans, and 1 of mixed race. This study demonstrates the wealth of information available in busy genetics clinics having cytogenetics laboratory as part of the genetic program.

Disruption of the gene Euchromatin Histone Methyl Transferase1 (Eu-HMTase1) is associated with the 9q34 subtelomeric deletion syndrome. *T. Kleefstra¹, M.J.G. Banning¹, H. van Esch², W. Nillesen¹, A.R. Oudakker¹, A. de Brouwer¹, H.G. Yntema¹, N. de Leeuw¹, B.C.J. Hamel¹, E.A. Sistermans¹, C. van Ravenswaay¹, M. Smidt³, H.G. Brunner¹, B.B.A. de Vries¹, H. van Bokhoven¹.* 1) Human Genetics, UMC ST Radboud, Nijmegen, Netherlands; 2) Centre for Human Genetics, University Hospital Leuven, Belgium; 3) Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, The Netherlands.

Submicroscopic subtelomeric deletions have recently been reported to account for ~ 5% of the total population of mental retardation. Some submicroscopic telomeric deletions are rather common which allow recognition of a specific phenotype. Recently, several patients have been reported with a terminal submicroscopic deletion of chromosome 9q. Common features seen in these patients are severe mental retardation, hypotonia, brachycephaly, flat face with hypertelorism, synophrys, anteverted nares, thickened lower lip and carp mouth with macroglossia and conotruncal heart defects. The minimal critical region responsible for this 9q subtelomeric deletion phenotype (9q-), has been previously estimated to be ~1 MB. We have detected an interstitial deletion 9q34.3 in a patient with a phenotype that is clearly different from the 9q- deletion phenotype. The distal endpoint of this interstitial deletion seems to be close or overlapping with the common breakpoint region in 9q- deletion patients, suggesting a more distal localization of the causative gene(s) for the 9q- phenotype. In a second patient, we characterized the breakpoints of a de novo balanced translocation t(X;9)(p11.23;q34.3). This patient is a mentally retarded female patient with features similar to those seen in the 9q- phenotype. Molecular investigations revealed that the chromosome 9 breakpoint disrupted the gene Euchromatin Histone Methyl Transferase 1 (Eu-HMTase1), situated in the refined critical region at the telomeric end of chromosome 9q. Expression data suggest that Eu-HMTase1 is highly abundant in brain and heart tissue and is likely to play an essential role in early embryonic development. These data strongly suggest that haploinsufficiency of Eu-HMTase1 is responsible for the 9q- phenotype.

Interstitial deletion of 13q associated with polymicrogyria. *J.M. Kogan¹, J.C. Egelhoff², H.M. Saal¹*. 1) Division of Human Genetics, Cincinnati Childrens Hospital Medical Center, Cincinnati, OH; 2) Department of Radiology, Cincinnati Childrens Hospital Medical Center, Cincinnati, OH.

Deletion of the long arm of chromosome 13 is a rare condition characterized by a range of clinical findings. We report a male dizygotic twin with a karyotype of 46,XY,del(13)(q14.1q31.2). His clinical findings include failure to thrive, hypotonia, polymicrogyria, bilateral foci of retinoblastoma, a mild hearing deficit, bilateral inguinal hernias, a submucous cleft palate, and dysmorphic features including a triangular shaped face, a small chin, protuberant eyes, downslanting palpebral fissures, and a downturned mouth. Chromosome analysis revealed an interstitial deletion of chromosome 13 which was confirmed by FISH analysis to include the Rb locus, but spare the 13q subtelomeric region. The karyotype was 46,XY,del(13)(q14.1q31.2).ish del(13)(RB1-,D13S327+) *de novo*. The parents karyotypes were normal. Retinoblastoma tumors are a well known complication of deletion of the retinoblastoma susceptibility gene, located at chromosome 13q14.2. Growth retardation is another common feature that has been described in other patients with a deletion of 13q. At 4 months of age this patient had brain findings on MRI consistent with bilateral frontal polymicrogyria. Another MRI at 13 months of age revealed additional involvement of the temporal lobes and left parieto-occipital cortex, as well as prominent infratentorial and supratentorial vasculature. Polymicrogyria is a disorder of neuronal migration resulting in cortical malformation characterized by numerous and shallow sulci. There are a variety of polymicrogyria syndromes that are distinguished by the cortical location of the abnormal folding. Several of the subtypes have known genetic loci associated with them. To our knowledge, this is the only published case of polymicrogyria in association with a deletion of chromosome 13. Additional studies need to be done to further characterize the relationship between chromosome 13q and polymicrogyria. Nevertheless, these findings reinforce the need for chromosome analysis in any patient with polymicrogyria.

Questionnaire-based comprehensive study of infants with trisomy 18 in Japan. *T. Kosho*¹, *H. Kuniba*², *Y. Tanikawa*³, *Y. Hashimoto*⁴, *H. Sakurai*⁵, *Y. Fukushima*¹. 1) Dept Medical Genetics, Shinshu Univ Sch Medicine, Nagano, Japan; 2) Department of Pediatrics, St.Barnabas' Hospital, Osaka, Japan; 3) Kobe City College of Nursing, Hyogo, Japan; 4) Sanno Institute of Psychology, Tokyo, Japan; 5) Trisomy 18 Support Group in Japan, Nagano, Japan.

Management of infants with trisomy 18 has been controversial, though it is the second most common autosomal trisomy in liveborn infants. Insufficient data about the natural history are one of the most important factors. We conducted a questionnaire-based comprehensive study of infants with trisomy 18 in Japan to help delineate the natural history. We collected data about pregnancy, delivery, complications, interventions, growth, development, and parental narratives from 88 families (the response rate was 70%) in Trisomy 18 Support Group in Japan. 58 % of the babies were delivered by C-section. Mean gestational age was 38 weeks and 5 days and mean birth weight was 1869g. Survival rate was 80 % at 1 month and 31 % at 1 year. Major cause of death was heart failure due to congenital heart diseases (53%). Convulsion, observed in 20 % of the infants, disappeared or improved in 35 %, was stable in 50 %, and worsened in 15 %. In 5 long-term survivors over 10 years old, 4 accomplished fully oral feeding and 3 walking alone. 54 % could go home. In those receiving respiratory supports (50 %), 45 % could withdraw them. 18 operations including 5 cardiac surgeries were performed. While 32 % of the families were presented by medical staffs the principle that the babies would be treated as intensively as possible, 39 % were offered some limitations of the therapies. From parental narratives, many felt happy with the babies birth and existence themselves. They also felt dissatisfaction with the stereotyped opinions from many medical staffs that any aggressive treatments would not be recommended without any sufficient informations about daily lives of the survivors and activities of the support group. Though considering inevitable ascertainment bias and inaccuracy in collecting data, the results will provide informations useful in counseling families of fetuses and infants with trisomy 18.

CHROMOSOME 4 IN THE GENETIC CLINIC. *B. Kousseff.* Pediatrics & Genetics, USF Regional Genetics Prog, Tampa, FL.

Between 1/2/81 and 12/31/02 through busy (2002 patient volume 2,797) prenatal and pediatric genetics clinics at the University of South Florida 42,795 probands/families were evaluated. Forty-nine had anomalous chromosome 4. Nineteen had translocations; 3 each had t(1;4) and t(4;11); all with different break points. One t(4;11) was mosaic. Two each had t(3;4), t(4;5),t(4;9),and t(4;12); all with different breakpoints. One each had t(2;4), t(4;6), t(4;8), t(4;10), and t(4;16). Four were maternal, 3 were paternal, 4 were denovo and 8 were with incomplete parental studies. Four had complex chromosome rearrangement (CCR): two each had 4 and 3 breakpoints. Two were with paternal translocations, 1 with t(4;7), and t(13;18), and another with t(1;4) and ins(4). There were 10 probands with del 4p; 3 denovo and 7 with incomplete parental studies. They demonstrated the varied phenotype of Wolf-Hirschhorn syndrome based on the size of the deleted segment of 4p. The 5 inversions were 3 paracentric and 2 pericentric. Among the paracentric there was 1 denovo and 2 with incomplete parental studies. The 2 pericentric were 1 maternal and 1 paternal. There were 3 dup 4p all denovo with breakpoint at p16. The 3 del 4q were one denovo, del 4q32 and 2 interstitial with incomplete parental studies. Both dup 4q were interstitial and without parental studies. There were 2 denovo ring (4) and 1 trisomy 4 that resulted in a miscarriage. Thirteen probands were prenatal and 36 were evaluated through the pediatric clinic. Thirty-nine were Caucasians, 5 Hispanic, 4 African-Americans, and 1 Chinese. This study demonstrates the wealth of information available in busy genetics clinics having cytogenetics laboratory as part of the genetic program.

A case of 20p trisomy syndrome resulting from a tandem duplication of whole short arm of chromosome 20. *H. Maruyama, T. Satoh, M. Shinozuka, S. Ninomiya, Y. Yokoyama, T. Morishima.* Department of Pediatrics, Okayama University Graduate School of Medicine and Dentistry, Okayama, Okayama, Japan.

(Introduction) Trisomy 20p is rare and 54 cases were reported in our knowledge. Major parts of trisomy 20p are the product of reciprocal translocations. A few cases are caused by parental chromosomal inversions. We report the first case of 20p trisomy syndrome resulting from tandem duplication of whole short arm of chromosome 20. (Clinical features) The proband, a 1-year-and-4-month-old girl, was referred to us for the evaluation of multiple congenital malformations. The infant was born at 40 weeks of gestation. The birth weight was 3949g. Physical examination showed flat occiput, coarse hair, round face, upslanted palpebral fissure, hypertelorism, prominent anteverted nose, low set ear, simian crease, webbed neck, thorax deformity, cubitus valgus, hypotonia, delayed dental eruption and developmental delay. Echocardiogram showed ventricular septal defect. The computed tomography of the brain revealed agenesis of corpus callosum. (Cytogenetic analysis) Peripheral blood lymphocytes were cultured from the patient and both parents. GTG banding of the patient showed that an unknown origin chromosome was added to the short arm of chromosome 20. Spectral karyotyping showed the extra material to be of chromosome 20 origin. So we diagnosed that the karyotype of this patient was 46, XX dup(20)(p11.2p13). The parents had normal chromosomes. (Conclusion) A trisomy 20p syndrome has been difficult to describe as many cases involve only partial trisomy, often with partial monosomy of the partner chromosome. As our patient is an example of pure trisomy 20p, the features are important in helping to delineate the syndrome. In our case, agenesis of corpus callosum was found. So it might be a new feature of trisomy 20p.

Multiple congenital anomalies in a mexican patient with trisomy X syndrome. *P. Medina, S. Monroy, V. del Castillo, R. Cruz, L. Gomez, A. Gonzalez del Angel.* Department of Human Genetics, Instituto Nacional de Pediatría, Mexico city, Mexico.

The trisomy X syndrome is caused by nondisjunction at maternal meiosis I. It is observed in approximately 1 in 1000 newborn females. Women with 47,XXX karyotype often have a normal phenotype with relatively tall stature and normal pubertal development, hormone levels, and reproductive competency. In some patients multiple congenital anomalies have been described as malformations of the urogenital tract, brain, skeleton, heart, pulmonary hypoplasia and craniofacial dysmorphisms such as short palpebral fissures, midface hypoplasia, long philtrum, square chin and abnormal ears but without a consistent phenotype. They are at risk for developmental delay, including language and learning impairment, mild global intellectual deficits and psychiatric disturbances as schizophrenia. We report an 8 year old female, her parents were healthy without history of consanguinity, and she had 3 healthy sisters and a healthy brother. She had mild global intellectual deficits, minor dysmorphisms such as short palpebral fissures, retrognathia and a broad nose. By echocardiogram a displasic pulmonary valve, persistent arterial conduct and tricuspid deficiency were detected. Abdominal ultrasonography showed right ectopic kidney and extensive collector system. Ophthalmologic evaluation detected a left iris colobome and bilateral dacryostenosis. Karyotype analysis of lymphocyte and fibroblast cells showed 47, XXX in all metaphases examined. The ophtalmologic abnormalities seen in our patient have not been previously described in trisomy X syndrome. In fact, the congenital anomalies that have been reported in patients with 47, XXX karyotype may be coincidental or they can be etiologically related to the aneuploidy. Establishing genetic counseling and a direct relationship between congenital anomalies and 47.XXX remains difficult, as in the present case, especially because of the lack of a distinct phenotype. Further clinical data are needed to resolve this question.

Platelet dysfunction in a patient with 11q24.2 monosomy. *D. Melis¹, L. Pecoraro¹, F. Majo¹, R. Genesio², A. Conti², M. Tardi¹, D. De Brasi¹, V. Barletta¹, F. Fabbrini², R. Della Casa¹, G. Andria¹, G. Sebastio¹.* 1) Dept Pediatrics, Federico II Univ, Naples, Italy; 2) Dept Biology, Molecular and Cellular Pathology, Federico II Univ, Naples, Italy.

Jacobsen syndrome is characterized by psychomotor and growth retardation, microcephaly, trigonocephaly, facial dysmorphism, thrombocytopenia, eye and heart anomalies. We describe a patient with 11q24.2 deletion. At birth, thrombocytopenia was observed; percentage of chromatide breaks and bone marrow analysis were normal. She was admitted every 6 months until she was 5 years old. Physical examination showed mild mental retardation, short stature, clinodactyly and dysmorphic features including relative macrocephaly, downslanting palpebral fissures, hypertelorism, broad flat nasal bridge, short nose, carpe-shaped mouth, short neck. The mean platelet volume and the platelet count were checked periodically during the follow-up and a cyclic pattern was observed. A low platelet count with normal mean platelet volume was observed in the first year of life; mean platelet volume was found to be increased afterward. Direct and indirect test for platelet antibodies were negative. Peripheral blood smear, performed at the latest control, showed few giant platelets. A reduced platelet aggregation after exposure to ADP and collagene was scored. A low-normal aggregation capacity was also observed after exposure to ristocetin and arachidonic acid. The phenotype of the six patients with 11q24.2 deletion described in the literature is characterized by short stature, macrocephaly, mild mental retardation, hypertelorism, carpe-shaped mouth, broad flat nasal bridge, short nose, short neck and clinodactyly, not including trigonocephaly, microcephaly, severe mental retardation, ocular and cardiac malformation, which are typical of Jacobsen syndrome. By analyzing the phenotype of these patients it is possible to hypothesize a typical phenotype for the monosomy 11q24.2. No systematic study of thrombocytopenia and platelet function has ever been performed in the patients with 11q24.2 deletion. The detection of platelet dysfunction could suggest that a gene involved in platelet function is present at 11q24.2.

Alagille syndrome resulting from an apparently balanced de novo translocation: 46,XX,t(1;20)(p22.1;p11.2). R. Segel¹, J.M. Cowan¹, R.P. Spiro², L.A. Demmer¹. 1) Pediatrics, Tufts Univ. School of Medicine, Boston, MA; 2) National Birth Defects Center, Waltham, MA.

Alagille syndrome (AGS; McKusick no. 118540) is an autosomal dominant developmental disorder affecting multiple organ systems with variable expressivity. Alagille syndrome was mapped to 20p11.2-p12.2 and subsequently *JAG1* mutations and gene deletions were demonstrated in AGS. Although it has been suggested that AGS patients with large deletions may be more prone to developmental delay and hearing loss, no clear genotype-phenotype correlation has been identified for AGS gene mutation or deletion carriers. To date, two cases of AGS associated with reciprocal translocations have been reported. The first patient was found to have a 2-3Mb deletion at the chromosome 20 breakpoint and had the typical facial, liver, cardiac, skeletal and eye findings described in AGS, as well as FTT and microcephaly, but no developmental delay. Her father and sister also carried the translocation but were less severely affected. The second patient was found to have a >3Mb deletion which included *JAG1* and presented with typical dysmorphic features and liver disease, complex congenital heart disease, heart failure, and poor weight gain. We present a new case of AGS arising as a result of a de novo translocation. Our patient has the typical facial features of Alagille syndrome, VSD, PDA, direct hyperbilirubinemia, elevated liver enzymes, posterior embryotoxon, and a butterfly vertebra. Hearing screen was normal, as was renal ultrasound. Growth and development are normal at 8 months. The pregnancy was achieved via IVF/ICSI with fresh sperm 18 months after the father had completed chemo/radiotherapy to the groin for non-Hodgkin's lymphoma. Parental karyotypes were normal.

Future counseling for this couple presents several challenges. There is an accumulating literature suggesting an increased frequency of chromosome anomalies in offspring conceived via ICSI. In addition this father's lymphoma treatment increases his risk of producing chromosomally abnormal sperm. This couple should be offered prenatal diagnosis in all future pregnancies.

Tandem duplication of chromosome 7q11.22-q21.11 in a patient with developmental delay. *E. Seo¹, N.E. Lanphear², S.W. Scherer³, L.R. Osborne⁴.* 1) Medical Genetics Clinic & Lab, University of Ulsan, Asan Medical Centre, Seoul, Korea; 2) Pediatrics, Cincinnati Childrens Hospital Medical Center, Cincinnati, OH; 3) Genetics and Genomic Biology, Hospital for Sick Children, Toronto, Ontario, Canada; 4) Medicine, University of Toronto, Toronto, Ontario, Canada.

Duplication of the long arm of chromosome 7 is very rare and the clinical presentation is extremely heterogeneous, depending on extent and composition of chromosome bands included within the duplication. We present the first patient to be reported with a confirmed duplication of the chromosome region 7q11.22-q21.11 including the interval commonly deleted in Williams-Beuren syndrome (WBS). This male patient presented at the genetics clinic at the age of 7 months with hypotonia and dysmorphic features. The patient is now 18 months old and is at 50th centile for weight and height with a head circumference in the 10-25th centile. Echocardiogram was normal and there was no evidence of involvement of the eyes or kidneys. He has developmental delay, with greatest deficits in gross motor skills and oral motor skills, including expressive language. His facial features are not typical of WBS with low set ears and a very small mouth, and he has no vocalization pattern and has not yet been weaned from the bottle. Karyotyping revealed a tandem duplication of 7q11.23-q21.2 that was confirmed by fluorescence in situ hybridization with probes spanning the region. The duplication was shown to extend approximately 16.5 Mb from RP11-325K1 to RP11-665O4. Both his parents had normal karyotypes and FISH analysis did not show a duplication. The clinical characteristics of this patient are different from those of WBS and suggest that individuals presenting with WBS-like features are unlikely to carry a duplication of 7q11.23. This study provides new information for the clinical characterization of duplications of chromosome 7q11.22-q21.11.

Familial tandem duplication of chromosome 20p. A. Shanmugham¹, S. Gupta², P. Koduru², A. Yenamandra³, L. Mehta¹. 1) Schneider Children's Hospital at North Shore; 2) Cytogenetics Laboratory, North Shore University Hospital, Manhasset, NY; 3) Vanderbilt University, School of Medicine.

Duplication of chromosome 20p is a rare abnormality described in association with developmental disabilities and birth defects. We present an 8 year old girl with a history of learning disabilities, strabismus and bifocal vision abnormalities, short stature, relative macrocephaly and subtle facial dysmorphisms, including a round face with micrognathia and prominent forehead. She is classified as learning disabled with global delays, most marked in fine motor performance and visual motor skills. Chromosome analysis identified a chromosome 20p duplication [46,XX,dup(20)(p11.2p12)]. Parental blood chromosome analysis identified the father of the patient to have the same duplication [46,XY,dup(20)(p11.2p12)]. Proband's father also reported learning disabilities and short stature. He also has minor facial dysmorphisms and no medical problems. Chromosome 20p duplication is mainly reported in association with unbalanced chromosome translocations where there is aneuploidy for other chromosome segments. This patient and her father carry a rare tandem duplication. To our knowledge, only one other familial tandem duplication of chromosome 20p has been reported (Moog et al, 1996). Reported findings of partial 20p trisomy are moderate mental retardation with speech delays and poor motor coordination. Heart, renal and vertebral defects have also been reported. A recognizable facial phenotype has been suggested with a round face, prominent cheeks, short nose, dental abnormalities, and coarse hair. Our patients present with a milder phenotype with regard to cognitive abilities and birth defects. This may be due to the lack of other associated chromosome imbalances as found in unbalanced translocation outcomes. Further cases of tandem 20p duplications are required to establish a more definitive phenotype.

A new microdeletion syndrome associated with congenital diaphragmatic hernia at chromosome 15q26. *A.M. Slavotinek¹, M. dela Cruz², S.S. Lee¹, R. Davis³, A. Shrit⁴, E. Carlson², D. Albertson³, D. Pinkel³.* 1) Dept Pediatrics, UCSF, San Francisco, CA; 2) Genome Core Facility, UCSF, San Francisco, CA; 3) Comprehensive Cancer Center, UCSF, San Francisco, CA; 4) Dept. Pathology, Miami Valley Hospital, Dayton, OH.

Congenital diaphragmatic hernia (CDH) occurs in 1 in 2500 live births and is associated with a high perinatal mortality. The genetic etiology of this sporadic malformation remains unknown. Chromosome abnormalities have been reported in 10-33% of patients and these cases frequently have additional anomalies. We are currently using array comparative genome hybridization (array CGH) to detect submicroscopic chromosome rearrangements in CDH with malformations suggestive of Fryns syndrome. In a deceased female infant who was small for gestational age and had CDH, pulmonary hypoplasia, cleft palate, hypoplastic left heart syndrome, a single umbilical artery and talipes, we found reduced copy number for two BAC clones at chromosome 15q26.2. Microsatellite marker analysis showed a deletion of the maternal allele for D15S1014 mapped to 15q26.2, confirming an interstitial deletion estimated to be between 4 and 10 Mb in size. A karyotype at 400-band resolution had shown an apparently normal female karyotype (46, XX). There are 4 previous reports of CDH and pure monosomy for chromosome 15q24 to 15qter and the critical region for a gene involved in diaphragm formation has been hypothesized to be between chromosome 15q26.1 and 15q26.2. Clinical findings besides the hernia have included reduced growth parameters, minor craniofacial dysmorphism, congenital heart disease, pulmonary hypoplasia, renal hypoplasia, fifth finger brachydactyly and clinodactyly, talipes equinovarus and a single umbilical artery. We consider that these anomalies constitute a new and recognizable contiguous gene deletion syndrome associated with CDH at chromosome 15q26. Our case is the first report of CDH associated with a submicroscopic deletion and we consider that testing for deletions of band 15q26 is indicated for CDH with additional malformations consistent with 15q26 deletion syndrome.

A new case of terminal deletion of the long arm of chromosome 13 revealed by subtelomeric FISH-analysis. *M.I. Soller¹, G. Blennow²*. 1) Clinical Genetics, Lund University Hospital, Lund, Sweden; 2) Pediatrics, Lund University Hospital, Lund, Sweden.

We report on a 1-year-old boy with mild to moderate mental retardation with speech retardation, microcephaly, mild hypertelorism, short philtrum and malformed ears. The high-resolution G-band karyotype was normal. Fluorescence in situ Hybridization (FISH) with specific probes of chromosomal telomeric regions (ToTelVysion Probe Panel) to metaphase spreads were then used and showed a terminal deletion of the long arm of chromosome 13, band q34 ((VIJ yRM2002, D13S327). The parents showed normal chromosomes. Several cases with deletions of distal parts of the long arm of chromosome 13 have been published, but only one previous case revealed an identical sole subtelomeric terminal deletion of 13q34. In both patients the phenotype was characterized by mild to moderate mental retardation, mild facial dysmorphism, microcephaly, low birth weight, and hypertelorism. The mild phenotype might explain why only two patients with this deletion have been described so far.

Characterisation of chromosomal deletions involving 3q26->3q28 using a novel method for Multiplex Ligation-Dependent Probe Amplification, and relation of deletion position to clinical phenotype. *R. Stern¹, C. Mackie Ogilvie¹, A. Male¹, D. Bonneau², A. Guichet², R. Roberts¹, J. Berg^{1,3}.* 1) Medical and Molecular Genetics, Guy's Hospital, London, United Kingdom; 2) Service de Gentique Medicale, CHU Angers, France; 3) Department of Molecular and Cellular Pathology, Ninewells Hospital and Medical School, Dundee, DD1 9SY, U.K.

A number of patients have been reported with a complex clinical phenotype caused by deletions involving chromosome 3q26 ->q28. The phenotypic features reported include anophthalmia/severe microphthalmia which is presumed to be due to deletion of SOX2. Additional features are seen, including microcephaly, a hypoplastic pituitary, intrauterine growth retardation (IUGR), midline brain defects, abnormal neuronal migration and unilateral absence of the first cranial nerve, which may be caused by contiguous deletion of other genes in the region. Multiplex ligation-dependent probe amplification (MLPA) is a technique for determining copy number changes at multiple target loci. We wished to use this technique to assess copy number changes in this region. This technique utilises two sequence tagged half-probes corresponding to the target loci, which are ligated together and then amplified by universal primers complementary to the sequence tags. However, the probe sets need to be generated by cloning in single-stranded bacteriophage vectors. To circumvent this time-consuming process, we successfully developed a novel approach using synthetic oligonucleotide probes to analyse these patients by MLPA. Our data from the analysis of two patients suggests that the minimum candidate interval for the genes causing microcephaly, IUGR and a small pituitary is approximately between 178.8 and 189.1MB (ensembl build v22), including the genes *chordin* and *dishevelled*, implicated in neuronal and eye development. The interval for absence of the corpus callosum, unilateral absence of the first nerve and abnormal neuronal migration is wider at 12.5 MB between 178.9 and 191.4MB, but may be restricted to the interval between 189.1 and 191.4MB. This region contains *claudin 1*, an integral membrane protein involved in tight junctions and expressed in the occipital lobe.

Costovertebral dysplasia caused by dup22(pter->q11.2). *V.S. Tonk¹, G. Wilson^{1,3,4}, R. Schutt², G. Wood¹, H. Wyandt⁵, M. Ito⁵.* 1) Department of Pediatrics, Texas Tech University, Lubbock, TX; 2) Department of Orthopedics, Texas Tech University, Lubbock, TX; 3) Cook Children's Hospital, Fort Worth, TX; 4) Texas Child Neurology, Plano, TX; 5) Center for Human Genetics and Department of Pediatrics, Boston University School of Medicine, Boston, MA.

Here, we report a female infant affected with costovertebral dysplasia (CVD) presented with subtle facial anomalies and neonatal respiratory distress. The child has had severe growth and developmental delay due to pulmonary insufficiency and hypoxia during 11 months of her life. Two prior reports (before banding technology) of D/G and D/D translocations associated with costovertebral dysplasia suggest that imbalance of a peri-centromeric region common to chromosomes 14 and 22 may cause this anomaly. Her karyotype demonstrated an extra NOR-positive fragment, and subsequently it was identified as chromosome 22 (pterq11.2pat) by fluorescent and DNA microsatellite marker studies. Our study strongly indicates a partial trisomy of chromosome 22 is a cause of CVD in this case.

Unbalanced subtelomeric rearrangement involving the terminal ends of chromosome 4 and 7: Case report, review of the literature and discussion of 4q deletion phenotype. *P. van Galen¹, M. Innes¹, J. Chernos², K. Bowser².*
1) Dept of Medical Genetics, Alberta Children's Hosp, Calgary, AB, Canada; 2) Cytogenetics Laboratory, Alberta Children's Hosp Calgary, AB Canada.

We report on a boy with a subtle chromosome 4q35-qter deletion initially detected by G band analysis at the 625-650 band level previously missed at 550-600 band level. Subsequent subtelomere FISH with a multi-telomere probe panel (ToTelvysion) showed the abnormal chromosome 4 to be derived from an unbalanced translocation between the distal long arm of chromosome 4 and chromosome 7 resulting in deletion of 4q35-4qter and duplication of 7q36-7qter. Parental chromosomes by G banding analysis were normal indicating a de novo event. Clinical findings include: mild developmental delay, ADHD, macrocephaly, hypotonia, V shaped cleft palate, small VSD, bilateral optic nerve hypoplasia, short palpebral fissures, epicanthal folds, short upturned nose, long simple philtrum, and small simple posterior rotated ears. Given the combination of cleft palate and VSD he had been previously diagnosed clinically with VCFS despite normal 22q11 FISH. We are aware of only one similar case of a familial cryptic translocation resulting in two brothers with deletion of 4q33-4qter and duplication 7q34-7qter. Similar features are limited to macrocephaly and ADHD suggesting limited involvement of the single band 7q36 duplication in our patient. Instead our case supports previous observations of a distinct phenotype associated with terminal deletion of chromosome 4 and validates a correlation between the severity of phenotype and amount of deleted chromosome. Common features of 4q terminal deletion syndrome include short nose with abnormal bridge, cleft palate, congenital heart defects, poor postnatal growth, hypotonia and moderate to severe MR. This case emphasizes the role of subtle subtelomeric rearrangements in the etiology of mild MR and the importance of preceding subtelomere FISH with high resolution G band analysis.

Speech and language impairment and oromotor dyspraxia due to deletion of 7q31 which involves FOXP2. S.

Zeesman^{1,2}, *M.J.M. Nowaczyk*^{1,2,3}, *I. Teshima*^{4,5}, *W. Roberts*⁵, *J. Oram Cardy*⁶, *J. Brian*⁵, *L. Senman*⁵, *S. Choufani*⁷, *L.R. Osborne*^{8,9}, *S.W. Scherer*^{7,8,9}. 1) Dept of Pediatrics, McMaster University Medical Center, Hamilton, ON, Canada; 2) Hamilton Regional Lab Medicine Program, Hamilton Health Sciences, Hamilton, ON; 3) Dept of Pathology and Molecular Medicine, McMaster University, Hamilton, ON; 4) Dept of Pediatric Lab Medicine, Hospital for Sick Children, Toronto, ON; 5) Dept of Pediatrics, University of Toronto and Hospital for Sick Children, Toronto, ON; 6) Dept of Medical Imaging, University of Toronto and Hospital for Sick Children, Toronto, ON; 7) Dept of Genetics and Genomic Biology, Hospital for Sick Children, Toronto, ON; 8) Dept of Medicine, University of Toronto, Toronto, ON; 9) Dept of Molecular and Medical Genetics, University of Toronto, Toronto, ON, Canada.

We report the clinical, cytogenetic and molecular findings in a girl with a deletion of chromosome 7q31-q32. This child has a severe communication disorder with evidence of oromotor dyspraxia, dysmorphic features and mild developmental delay. Notably, she is unable to cough, sneeze or laugh spontaneously. Her deletion includes the FOXP2 gene, which has recently been associated with speech and language impairment and a similar form of oromotor dyspraxia in at least two other unrelated families. We suggest that the probands communication disorder and oromotor deficiency are due to haploinsufficiency for FOXP2 and that her dysmorphism and developmental delay are a consequence of the absence of the other genes involved in the microdeletion. Taken together with results from the literature, our findings suggest that the child's presentation may define a new contiguous gene deletion syndrome encompassing the 7q31-FOXP2 region. Cytogenetic and molecular analysis of this region should be considered for other individuals displaying similar characteristics.

13q critical region for anorectal and urogenital anomalies. *A.R. Zinn¹, L.J. Santos¹, R.A. Schultz², N.M. Garcia³, L.A. Baker⁴.* 1) McDermott Center for Human Growth & Development, UT Southwestern Med School, Dallas, TX; 2) Dept. of Pathology, UT Southwestern Med School, Dallas, TX; 3) Dept. of Surgery, UT Southwestern Med School, Dallas, TX; 4) Dept. of Urology, UT Southwestern Med School, Dallas, TX.

Introduction: The 13q-deletion syndrome includes severe genitourinary and anorectal birth defects. Bartsch et al. (Am J Med Genet 65:218, 1996) have suggested a critical region for these anomalies in the interval of 13q32.2-34. We sought to narrow this critical region, thereby delineating the causal gene(s) mediating these anomalies.

Methods: Nine patients with known deletions around 13q32-qter were ascertained. Peripheral blood was obtained from patients and available parents. Deletions were mapped by loss of heterozygosity of microsatellite markers and/or by fluorescence in situ hybridization (FISH) on patient lymphoblastoid metaphase preps. The data were synthesized and a deletion map defining the critical region was generated.

Results: The patients fell into three groups: 1) four patients with anorectal and/or genitourinary anomalies (hypospadias and/or penoscrotal transposition), 2) three male patients with developmental delay but no anorectal or genitourinary anomalies, and 3) two XY patients with ambiguous genitalia without anorectal anomalies. The proximal boundary of the critical region was delimited by marker D13S280 in 13q33.1.

Conclusions: The critical region of chromosome 13q mediating genitourinary/anorectal anomalies has been mapped to an 11 Mb interval. Haploinsufficiency of 13q shows incomplete penetrance for anorectal and genitourinary anomalies. There may be a second locus in 13q31.3 mediating ambiguous genitalia. Mapping additional deletions will narrow the critical region. Identification of the gene(s) mediating these syndromic genitourinary defects will likely further our knowledge of molecular mediators of nonsyndromic hypospadias, penoscrotal transposition and anorectal malformations.

Expanded phenotype of the 22q duplication syndrome. *S. Hassed, S.A. Vaz, J. Lee, J.J. Mulvihill, S. Li.* Dept Pediatrics/Genetics, Univ Oklahoma Medical Ctr, Oklahoma City, OK.

The velocardiofacial, DiGeorge, and Sphrintzen syndromes result from microdeletions in a critical region on chromosome 22q and, hence, are collectively termed the 22q deletion syndrome. Like other microdeletion syndromes with a paired condition due to duplication of the same genomic region, the 22q deletion syndrome has a complementary 22q duplication syndrome, represented by xx published cases with mild craniofacial anomalies and slight developmental or cognitive delay. To add to the spectrum of the 22q duplication phenotype, we report an 18-year-old female with chronic aortic regurgitation, mild mitral and tricuspid regurgitation, recurrent renal infections, kidney stones, and pyelonephritis resulting in surgery to remove scar tissue and reimplant ureters,, hypernasal speech, normal intelligence, and features of Asperger syndrome. Family history revealed her mother had hypernasal speech and no other health problems. A brother had severe strokes in his 20s, due to cerebrovascular hemorrhage. The patient had 46,XX karyotype in all blood cells, and FISH studies with TUPLE2 showed duplication of 22q identified on interphase cells. The 22q duplication syndrome may not be so benign a condition as previously described and may share features with 22q deletion syndrome. Again, it is suggested that, in all FISH studies, interphase cells be scanned along with the metaphase cells, since duplications are much more obvious in interphase cells than in metaphase cells.

Y chromosome sequences in two patients with true hermaphroditism. *J. Toscano-Flores^{1,2}, M. Diaz-Rodriguez^{1,2}, J.R. Padilla^{1,2}, F. Rivas¹, M.L. Ramirez-Duenas¹.* 1) Western Biomedical Research Center, Instituto Mexicano del Seguro Social; 2) Graduate Studies Program in Human Genetics, Universidad de Guadalajara, Guadalajara, Mexico.

True hermaphroditism (TH, OMIM #235600) is a rare dysfunction (1:20,000 newborns) of sex differentiation, in which ovarian and testicular tissues coexist in one or both gonads. The most common karyotype is 46, XX, and few cases are familial. Two newborn patients from healthy and non-consanguineous parents with TH are presented here. Both cases had a 46, XX karyotype (>16 cells analyzed per case) on cytogenetic analysis. The first patient was evaluated at two months of age for ambiguous genitalia. In addition, physical examination showed asymmetric gonads (only one palpable in scrotum), small penis, and urogenital sinus. The second, a 6 month-old patient presented with a 2.5 cm phallus with an open urethral fossa, perineo-scrotal hypospadias, and no palpable gonads in the scrotum. Histopathologic studies revealed the presence of normal left ovary and right ovotestis in patient 1, and bilateral ovotestis in patient 2. Y chromosome sequences (STR markers: DYS390, DYS391, DYS392, DYS393 and DYS19), detected by PCR PAGE, were positive in both patients. Ambiguous genitalia in a newborn is a situation of social urgency, that requires immediate intervention. In the present cases, in addition to contributing to precise diagnosis and surgical treatment, positive Y sequences allow for a better classification of TH cases. Since these Y STRs span throughout the Y chromosome, the presence of hidden mosaicism is concluded. This approach can prove useful in the practice of clinical genetics.

Chromosome 1p36 deletion: A case with multiple anomalies overlapping VATER association. *M.G. Bialer¹, S. Race¹, R. Schiff¹, R. Smith¹, D. LaGrave², J. Neidich², B. White², A. Anguiano².* 1) Dept Pediatrics, North Shore LIJ Health System, Manhasset, NY; 2) Quest Diagnostics-Nichols Institute, San Juan Capistrano, CA.

The 1p36 deletion syndrome is the most common chromosome abnormality detected by subtelomeric FISH screening, with estimates of prevalence as high as 1 in 5,000. Cases typically have cognitive delay, dysmorphic features and congenital anomalies, most commonly congenital heart disease and cleft lip (CL)/palate. We present a case of 1p36 deletion with several previously unreported anomalies resulting in overlap with VATER association. Comprehensive U/S during the pregnancy revealed bilateral choroid plexus cysts (CPC) and a unilateral CL. Amniocentesis chromosomes, AFP, 22q11 FISH and fetal echo were reported to be nl. However, after birth, Ped. Cardiology evaluation revealed fenestrated foramen ovale and multiple muscular VSD's (swiss cheese septum) with large LR shunt. Genetics evaluation at 27 days revealed left thumb hypoplasia, complete left CL and alveolus, large anterior and posterior fontanelle and shawl scrotum. Renal U/S was nl. Vertebral X-rays revealed a butterfly vertebra at T7. Hand X-rays revealed hypoplastic 1st metacarpals and phalanges bilaterally. At this time the diagnosis of VATER association was considered, as there were 3 typical anomalies and other features were compatible. A blood chromosome analysis and DEB-stimulated chromosome breakage study were nl. When the patient developed seizures at 2 mo and was diagnosed with a mixed hearing loss, the diagnosis was reconsidered. Subtelomeric FISH screening was sent and revealed del(1)(p36.3). Parental subtelomeric FISH testing was nl. This case has 3 anomalies that we believe are previously unreported in 1p36 deletion syndrome: CPC, butterfly vertebra and thumb hypoplasia. Although the usual indication for subtelomeric FISH screening has been cognitive delay, this case was ascertained because of multiple congenital anomalies, seizures and hearing deficit. Subtelomeric FISH screening should be contemplated if a diagnosis of exclusion is being considered, e.g. VATER or CHARGE association, especially if there are atypical features.

Down Syndrome: twenty eight mexican patients from a second level Childrens Hospital. *L. Cornejo^{1,2}, E. Medecigo¹, L. Islas¹, R. Perez¹.* 1) Genetics, Hospital del Niño DIF, Pachuca de Soto, Hidalgo, Mexico; 2) Area Academica de Medicina, Instituto de Ciencias de la Salud, Universidad Autonoma del Estado de Hidalgo, Pachuca de Soto, Hidalgo, Mexico.

Introduction. There has been multiple reports about Down Syndrome from divers populations as the most frequent chromosomal alteration but from Hidalgo State, Mexico. These disease is the most frequently seen in our Genetic Department. Objective: To report the first large series of Down Syndrome patients from our Hospital focus in: medical demand attention timing, maternal and paternal age at the conception time, karyotyping results, and associated malformations. Material: Twenty eight patients attended at the Hospital from April 2003 to March 2004. The patients were clinically and karyotyping tested. They were attendent also at least in the Cardiac and Endocrinology departments. Results: Twenty seven patients were isolated cases (96%); sixteen patients were females (57%), and twelve patients were males (42%). Eighteen patients (64%) has been assisted at the hospital in their first year of life, the mean for this group was 2.8 months old; for those patients assisted after, the mean was 6 years old, two years old the youngest and 15 years old the oldest. October was the mode of the month to be born with six cases. The mean for maternal age was 30 years old, 19 years old the youngest and 42 years old the oldest; the mean for paternal age was 34 years old, 19 years old the youngest and 64 years old the oldest. Second gestation was the mode for gravity with six cases. We found fifteen cases out of eighteen (83%) with cardiac anomaly, and the PCA as the most common alteration. All isolated cases showed a regular 21 trisomy. All patients were normal for thyroid function. Two cases (7.17%) had imperforate anus, one of these was born from a diabetic mother; one patient (3.57%) had bilateral hearing loss; and, one patient (3.57%) had seizures. Conclusion: Maternal age as risk factor starts earlier than general population, cardiac (but malformation type)malformations are higher and endocrinology function is lower than the generally reported. We will look for a cross-sectional study in near future.

Implications of dup(8)(q24.1qter) and/or del(15)(q26.1qter) in Fryns syndrome. *C. Higgins, G.B. Schaefer, Z. VanDyke, T. Hempel, D. Pickering, M. Nelson, W.G. Sanger, B.J. Dave.* Univ Nebraska Medical Ctr, Omaha, NE.

We present a case of a deceased newborn male with a der(15)t(8;15)(q24.2;q26.2) and the clinical manifestations suggestive of Fryns syndrome. The infant was the product of a complicated pregnancy of a 38 year old primigravid female. Prenatal assessment revealed IUGR, hypoplastic left heart with a small VSD, hydronephrosis, a two-vessel cord, club feet, oligohydramnios, and a suspected congenital diaphragmatic hernia (CDH). Amniocytes revealed a 46,XY karyotype. The infant was born at term with a low birth weight and poor APGAR scores. Clinical exam shortly after birth confirmed the prenatal findings including CDH. Additionally, the infant had low-set, malformed pinnae, a flat nasal bridge, prominent forehead, anteverted nares, retromicrognathia, a microphallus and no palpable testes. The digits were angulated with hypoplastic nails. These findings were suggestive of Fryns syndrome. Cytogenetics, FISH, CGH, and DNA microarray studies were performed postnatally on blood lymphocytes and skin fibroblasts. A der(15)t(8;15) was cytogenetically detected only on prometaphase chromosomes at a 750 band level. FISH studies on blood and fibroblasts using subtelomeric probes detected a deletion of terminal 15q, which was replaced by an 8q telomere. Subsequent CGH and DNA microarray further delineated the breakpoints. CGH demonstrated the loss of terminal 15q and gain of terminal 8q. However, the DNA microarray studies allowed a delineation of the breakpoints within 8 megabases. The microarray analysis revealed that the genes located in 15q26.1 remained intact whereas those located in 15q26.3 and the 15q telomere were deleted. Thus, a deletion of the genes distal to 15q26.1 and/or the duplication of the region distal to 8q24.1 may be implicated in the congenital abnormalities observed in some cases of Fryns syndrome. A combination of prometaphase analysis, FISH, CGH and DNA microarray investigations in this case with a cryptic chromosomal abnormality facilitated the localization of the breakpoints. This illustrates the importance of an ever-increasing need to use technologies beyond conventional cytogenetics in the diagnosis of congenital abnormality syndromes.

Genetic and molecular analysis of a large cohort of patients with syndromic and non-syndromic trigonocephaly - screening for loss of heterozygosity in 9p and 11q regions. *F.S. Jehee¹, D. Johnson⁴, K. Abe¹, L. Alonso³, N. Alonso², C. Kim², D. Cavalcante⁶, S.A. Wall⁴, S. Boyd⁵, E. Jabs⁵, A.O. Wilkie⁴, M.R. Passos-Bueno¹.* 1) CEGH, IBUSP, Brazil; 2) FMUSP, Brazil; 3) UNIFESP, Brazil; 4) Craniofacial Unit, Oxford, UK; 5) Johns Hopkins University, USA; 6) UNICAMP, Brazil.

Trigonocephaly is mostly caused by craniosynostosis of the metopic suture and is characterized by a triangular forehead. It occurs as an isolated or syndromic malformation and affects more males than females. Little is known about the genetic aetiology of trigonocephaly and different models of inheritance have been proposed. Environmental factors, such as valproic acid, hyperthyroidism and microcephaly can also induce trigonocephaly. Deletions in two chromosomal regions, del(9p22-24) and del(11q23-24), are associated with syndromic phenotypes and include usually trigonocephaly. To contribute to a better understanding of the genetic basis of metopic synostoses we evaluated a large cohort of patients with syndromic and non-syndromic trigonocephaly. Additionally, we performed a loss-of-heterozygosity analysis to detect microdeletions and further restrict the 9p and 11q candidate regions. Our sample consisted of 96 patients, 39 non-syndromic, 35 syndromic, 5 with secondary trigonocephaly and 17 without complete clinical data. Consanguinity and multiplex families were observed in 3.9% and 10.4%, respectively. Male preponderance was higher in syndromic patients (4.2:1) than in non-syndromic (1.8:1). The empirical recurrence risk for non-syndromic and syndromic trigonocephaly were 2.8% and 11.8%. These data suggest that syndromic and non-syndromic cases may have different aetiology. We believe that the main inheritance pattern for non-syndromic trigonocephaly is multifactorial and that there is a greater involvement of genes on the X chromosome in syndromic cases. We found 7 deletions in syndromic patients: 6 in 9p and 1 in 11q, so the detection rate in the syndromic group was 20%. We conclude that microdeletion screening for 9p22-24 and 11q23-24 should be offered to all cases of syndromic trigonocephaly. FAPESP, CEPID (jehee@uol.com.br).

Partial trisomy 16p de novo duplication of p11.2p13.1 in three unrelated patients. *K. Keppler-Noreuil¹, K. Mathews², S. Patil¹.* 1) Dept Pediatrics/Med Genetics; 2) Div. of Ped. Neurology, Univ. of Iowa Hosps & Clinics, Iowa City, IA.

Complete trisomy 16p has been described with a similar pattern of malformations, including low birth weight, severe developmental delay, seizures, characteristic facial features, cleft palate, CHDs, renal and GU anomalies. Partial trisomy 16p may involve different regions with variable clinical findings, but most reported cases involve the entire short arm, and often are associated with other chromosome imbalance in a translocation. We report here the clinical and cytogenetic findings of 3 unrelated cases with de novo duplication of 16p involving p11.2p13.1 and compare the findings with 5 other reported cases.

Case 1, a 6 yr old girl, was diagnosed at 2 years of age with developmental delay (IQ 60-70); microcephaly, short stature and GH deficiency; VSD, coarctation, and mitral stenosis; cerebellar hypoplasia; and craniofacial findings of hypertelorism, epicanthal folds, posteriorly rotated ears, broad nasal bridge with anteverted nares. Her karyotype: 46,XX,dup(16)(p11.2;p12). Case 2 presented at 2 yrs with global developmental delay, small stature, 1 seizure, normal HC, and craniofacial findings of high nasal bridge, up-slanting PFs, and prominent mouth. His karyotype: 46,XY,dup(16)(p11.2;13.1). Case 3, 4 yrs, had seizures, hypotonia, developmental delay, tremor, VUR, anteriorly placed anus, normal growth, and craniofacial findings of mild hypertelorism, and shallow orbits. Her karyotype: 46,XX,dup(16)(p12.2;p13.11).

These cases with similar duplicated regions involving 16p11.2p13.1 had in common mild developmental delay, seizures, short stature and craniofacial abnormalities. However, other anomalies were variable. The 5 other reported cases in the literature also involved 16p11p13, three having involvement of 16p11.2p12. The only consistent finding among the combined 8 cases was developmental delay, and >50% had seizures, hypertelorism, narrow palpebral fissures, large nasal bridge, and low set ears, these being milder, but reminiscent of cases with complete trisomy 16p. All cases lacked life-threatening medical complications.

Bone ossification study in patients with achondroplasia. *M.G. Arteaga-A^{1, 2}, A. Gonzalez-DA², V. DelCastillo², H. Ahumada³, R. Castillo⁴.* 1) Depto. Genetica, Facultad de Medicina, UANL, Monterrey, N.L., Mexico; 2) Depto. Investigacion en Genetica Humana,; 3) Depto. de Radiologia e Imagen,; 4) Subdireccion de Investigacion, Instituto Nacional de Pediatria, Mexico.

Achondroplasia is the most common chondrodysplasia, with a frequency of about 1 in 15,000. It is caused by a mutation of the FGFR3 gene on chromosome 4 (4p16.3), coding for the fibroblast growth factor receptor; the altered FGFR3 is constitutively activated with unregulated signal transduction through the receptor, resulting in an inappropriate cartilage growth plate differentiation and ultimately deficient endochondral growth. Major clinical manifestations are easy to recognize: disproportionate short-limb dwarfism, macrocephaly, midfacial hypoplasia, rhizomelic micromelia, trident hand with relatively short fingers, limitations of elbow extension, kyphosis at thoracolumbar junction in infancy. The clinical diagnosis must be supported by radiological features: large calvaria with prominent frontal; decreased size of the skull and the foramen magnum; decrease in the interpediculate distance from upper to lower lumbar spine; flat, rounded iliac bones; shortening of the tubular bones; square or oval radiolucent areas in proximal femur and humerus in infancy; retarded and irregular ossification in the knee region; disproportionate shortness of the humerus and lengthening of the fibula. After detect disturbance of the bone ossification in a few patients we found that there aren't radiographic standards of skeletal development for patients with bone dysplasias and we consider is necessary to find a better method to detect abnormalities in this process than the visual matching process of the hand. For determinate the possible bone maturation disturbance of these patients, we evaluated bone radiographies of 47 patients from both sexes, with achondroplasia, admitted to the Genetic department of the INP since 1991 to june 2003, using the method of Garn et al, which evaluate in percentiles 19-20 ossification centers, according to sex and age, using three radiographs: hand, foot and knee. After skeletal assessment, we correlated the clinical findings of medical files. We present and discuss the results.

Increased risk of cardiovascular disease in a patient with Marfan Syndrome and a homozygous C677T mutation of the MTHFR gene which has been associated with Homocystinuria. *D. Basel, M.W. Kilpatrick, P. Tsipouras.*
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We present a 39-year old man who was diagnosed clinically with Marfan Syndrome based on the Ghent criteria. Subsequent analysis of the Fibrillin gene (FBN1) led to the identification of a C2001T mutation in exon 15 predicting a phenylalanine for cysteine amino acid substitution at residue 623 (C623F). The patient required aortic surgery. Pre-operative testing identified elevated levels of plasma homocysteine leading to molecular analysis of the n(5,10)-methyltetrahydrofolate reductase (MTHFR) gene. A homozygous C677T mutation of the gene was detected. The primary gene involved in homocystinuria is cystathionine beta-synthase (CBS). However, mutations in the MTHFR gene have been implicated as a cause of homocystinuria. The C677T mutation produces a thermolabile enzyme with reduced activity resulting in increased levels of homocysteine. This finding is in keeping with a recent history of 2 thrombo-occlusive episodes in our patient. He has experienced deep vein thromboses and cerebrovascular accidents (CVA) involving his visual cortex bilaterally. The presence of MTHFR homocystinuria is a confounding factor in an individual with Marfan syndrome and it impacts on the cardiovascular management of this patient. The relative frequency of homozygous C677T mutations in the general population warrants screening for the presence of this mutation in patients with Marfan syndrome.

Clinical characteristics of NF2 associated ependymomas. *F. Nunes, J. Harrington, M. MacCollin.* Dept Neurology, Massachusetts General Hosp, Charlestown, MA.

Ependymomas have been reported in approximately 33% of neurofibromatosis 2 (NF2) patients. Because spinal tumors, including ependymomas, schwannomas, and meningiomas, occur in up to 90% of NF2 patients it is imperative to understand the natural history of these tumors before deciding on intervention. Thirty-nine patients meeting diagnostic criteria for NF2 and showing radiographic characteristics of spinal ependymomas were included in this study. Full record review included clinical data, imaging studies, surgical reports, pathology reports and mutational analysis of the *NF2* gene. All tumors showing radiographic characteristics of ependymomas were located in the spinal cord, with no supra-tentorial ependymomas occurring among these patients. Eighty-two percent of the patients showed intramedullary tumors at the level of the cervical cord and cervical medullary junction confirming that this is a site of predilection for these tumors in NF2 patients. Thoracic ependymomas were seen in 44% of the patients. Multiple tumors were seen in 66% of patients with coexisting cervical and thoracic tumors seen in 33%. Only seven of 39 patients (20%) required intervention because of tumor related symptoms, including limb weakness (50%), pain (33%), and gait disturbances (17%). Mutational analysis of the *NF2* gene in genomic DNA was performed in 25 unrelated patients, with frameshift and nonsense mutations present in 40% of the patients. Splice site alterations were found in 20% of the patients, and in 40% mutations could not be found. No missense mutation was seen in this cohort. Interestingly, in a large family with exon 12 splice site mutation cervical ependymomas were seen in 8 members. In conclusion, ependymomas in NF2 patients have the cervical cord and cervical medullary junction as a site of predilection, and seldom require intervention. Further work is needed to define the molecular events in the *NF2* gene or elsewhere which predispose to ependymoma development.

Anthropometric landmarks from 3dMD surface images: a study of accuracy and repeatability. *K. Aldridge¹, S.A. Boyadjiev Boyd², V.B. DeLeon¹, J.T. Richtsmeier¹*. 1) Dept Anthropology, Pennsylvania State Univ, University Park, PA; 2) McKusick-Nathans Institute of Genetic Medicine, Baltimore, MD.

Anthropometric landmarks are a powerful tool for the assessment and characterization of facial dysmorphology. However, the collection of quantitative data from young children, especially those with associated developmental disability, can be challenging. Here, we test the accuracy and repeatability of landmark data collected from 3-dimensional images obtained by 3dMD photogrammetry technology. 3dMD imaging captures surface information in 2 msec., permitting rapid and noninvasive data collection. We obtained two surface images from each of 7 adults and 8 children. 3D landmark coordinate data were collected from 20 precisely-defined facial landmarks by two observers in separate trials. Analysis of interlandmark linear distances calculated from these combined data allowed to test between-scan accuracy and within- and between-observer repeatability. Results indicated between-scan accuracy was good. However, landmarks on the jaw had a relatively high level of between-scan variance, likely explained by movement at the TMJ between scans. Also, trigion, on the ear, had relatively low accuracy and apparent poor resolution of tragus, attributed to the oblique angle at which the surface of the tragus is captured. Within- and between-observer repeatability was acceptable and showed similar results, with the highest repeatability at the canthic and lip folds and on mediolateral placement of midline landmarks. Relatively lower repeatability was observed at gonion and superoinferior placement of midline landmarks, particularly glabella. Low repeatability at these landmarks is comparable to that observed in other modes of anthropometric data collection. These results support the use of surface imaging for analysis of facial phenotypes. Data is captured instantly and provides highly accurate information about surface morphology. However, care should be taken in selecting appropriate landmarks. We demonstrate that precise landmark definition, including detailed instructions for visualizing and collecting landmarks, substantially improves both within- and between-individual repeatability.

The OEIS complex in two brothers born to consanguineous parents. *S. Ala-Mello, H. Laivuori.* HUSLAB
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The OEIS complex is a rare combination of malformations comprising omphalocele, exstrophy of the cloaca, imperforate anus, and spinal defects (Carey et al. *Birth Defects Orig Art Ser XIV(6B)* 1978;14:253-263). The etiology is probably heterogeneous. The higher incidence of the OEIS complex in monozygotic twins than in dizygotic twins suggests a possible genetic contribution to the occurrence of these defects. Most cases of the OEIS complex are sporadic, but it has recurred in two children from separate pregnancies born to non-consanguineous parents (Smith et al. *J Med Genet* 1992;29:730-732). Here we report the findings of two brothers with the features of the OEIS complex born to consanguineous Kurdish parents (first cousins). Their third child was born at 30 weeks of gestation weighing 1540g, and died within an hour after delivery. The necropsy showed postaxial polydactyly of fingers and toes, dilated renal pelvices, biliary atresia, anal atresia, short bowel, pulmonary segmentation defects, heart defects (transposition of great arteries, single ventricle), absent tracheal opening, and micrognathia. The karyotype was normal (46,XY). Their fourth child was born at 36 weeks of gestation weighing 2510 g. After delivery several defects were noted: exstrophy of the cloaca with hypoplastic external genitalia, imperforate anus, omphalocele, and club feet. Radiological examination showed defects at the thoracic vertebral bodies (VII, IX and XI). The ultrasound examinations of heart and brain were normal, but the left kidney was not visualised. The karyotype was normal (46,XY). The recent literature has demonstrated the variability in the OEIS complex (Keppler-Noreuil et al. *Am. J Med Genet* 2001;99:271-279). The findings observed in these two brothers have been previously described in the spectrum of the OEIS complex (Oxford Medical Databases). We suggest that autosomal recessive inheritance is likely within this family.

AMNIOTIC BANDS SEQUENCE WITH BILATERAL FACIAL CLEFTS AND ENCEPHALOCELE. LE.
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The amniotic deformity, adhesion, and mutilation (ADAM) sequence is characterized by amputations, limbs are the most commonly involved, but it may be associated with a wide spectrum of congenital anomalies. Its inheritance is not clear. We describe a 13-months-old male, product of the first pregnancy from a young nonconsanguineous couple, without important familial antecedents. Abortion threatening at 4 month of pregnancy because of urinary and vaginal infection. At 8 months the obstetric ultrasound detected encephalocele; labor was induced unsuccessfully. Vaginal delivery at 42 weeks of gestation. Birth weight was 2.4 kg, length not recorded and Apgar score 9-10. Physical examination showed weight 4,2 kg and length 72 cm. Head circumference was not measured, because of a parietal encephalocele of 10-15 cm, with alopecia in the top, narrow bifrontal cranial diameter, prominent metopic. Face with bilateral longitudinal cleft (Tessier: 4 and partial 5-10) through superior lip to nasolacrimal union, as a consequence it was formed a V shaped cleft palate and a communicating cavity between nose and mouth. The disruption continued through parietal bones to posterior fontanelle. Coloboma of eyelids, ulcerous lesion in right cornea and opacity in left cornea. Wide nasal bridge and the premaxilla was separated of the face by the cleft. Pectus excavatum, heart auscultation with holosystolic murmur and sinusal pauses. Hypertonia of extremities, bilateral adducted thumb; right leg had a circumferential constriction. Rest of the exploration was normal. The patient died at 19-months-old by respiratory infection, but autopsy was no performed. Karyotype was 46,XY and cranial computed tomography showed mild cortical atrophy and absent parietal bones. There are few reports of ADAM associated to encephalocele and bilateral symmetrical facial clefts, their affectation degree was not so severe, but with a shorter term survival than our patient. This is the first report, to our knowledge, with such an association; it could be explained by a fortuitous event, but the disruption of ADAM as the basic defect, for both encephalocele and facial clefts, can not be rejected.

Sirenomelia with bilateral absent radii and oligodactyly: a new syndrome or a variant of the VACTERL association? *H. Berger*¹, *S. Keating*², *S. Viero*², *M. Thomas*³, *D. Chitayat*³. 1) Obstetrics and Gynaecology, Mount Sinai Hospital, Toronto, Ontario, Canada; 2) Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario, Canada; 3) Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, Ontario, Canada.

Sirenomelia is a rare congenital malformation characterized by single or fused lower limbs and other anomalies. Invariably, it is associated with urogenital abnormalities resulting in severe oligohydramnios. The incidence of this disorder is 1:60000 - 1:100,000 births and in most cases the abnormalities are restricted to the lower part of the body. We reviewed all cases with sirenomelia detected at our center and report two cases with similar upper limb abnormalities. There is a debate regarding the etiology of sirenomelia with some suggesting that it is but a variant of the Caudal Regression Syndrome (CRS). Others have noted an association between VACTERL and sirenomelia. The presence of upper limb abnormalities in three of ten cases detected in our center could support this association. However, the finding of two cases, in families of the same ethnic background (Sri-Lankan), with the same pattern of upper limb abnormalities, raises the possibility that this is a previously unidentified syndrome.

Bladder exstrophy-epispadias complex: analysis of 232 families. S.A. Boyadjiev Boyd¹, G.H. Ashrafi¹, C.L. Radford¹, S. South¹, G. Stetten¹, Y. Lakshmanan², J.L. Dodson², J.P. Gearhart². 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Division of Pediatric Urology, The James Buchanan Brady Urological Institute, Johns Hopkins University School of Medicine.

Objective: To identify genetic and non-genetic factors contributing to the risk of bladder exstrophy-epispadias complex (BEEC). **Patients and Methods:** A total of 291 BEEC families were invited to participate in this study and 232 of those were recruited. Epidemiologic information was obtained from 151 families, and a detailed clinical genetic examination was performed on 94 probands. More than 500 DNA samples were collected from 183 families for molecular analysis. **Conclusions:** BEEC is a sporadic isolated birth defect with a recurrence risk well below 1%. Among patients, 95% were Caucasian, and males were more common in both the epispadias group (M/F=2.2, n=29) and the classic bladder exstrophy group (M/F=1.8, n=164). In the cloacal exstrophy group, however the sex ratio was close to unity (M/F=1.1, n=15). A statistically significant association with advanced parental age was observed ($p < 0.001$). Birth weight, gestational age, maternal reproductive history, and exposures to tobacco and alcohol, did not appear significantly different from the general population. Seven affected had anomalies outside of the BEEC spectrum, but none of probands had defined syndromes or developmental delays. A male patient with classic bladder exstrophy and an apparently balanced *de novo* translocation 46,XY,t(8;9)(p11.2;q13) was identified. The chromosome 9 breakpoint was mapped within *CASPR3*. Multiple copies of this gene flanking the heterochromatic region on chromosome 9 were identified by FISH. Molecular analysis of the *HLXB9*, which causes Currarino syndrome, did not detect mutations in the blood or bladder DNA of ten BEEC patients. Expression profiling of developing, normal, and exstrophic human bladder identified a group of 154 potential candidate genes for SNP based association studies of case-parent BEEC trios.

Small head circumference of newborns as a possible Chernobyl aftermath. *G. Lazjuk¹, I. Zatsepin¹, R. Khmel¹, P. Verger², E. Robert-Gnansia³, B. Gagnière⁴.* 1) Belarus Institute for Hereditary Diseases, Minsk, Belarus; 2) Observatoire Régional de la Santé PACA, Marseille, France; 3) Institut Européen des Génomutations, Lyons, France; 4) Institut de Radioprotection et de Sûreté Nucléaire, Paris, France.

It is a well known fact that even relatively small doses of in utero radiation exposure of the fetuses can induce microcephaly and mental retardation (UNSCEAR, 1988; D. Bard et al., 1997). To assess a possible teratogenic impact of Chernobyl accident, we analyzed the anthropometrical sizes of infants, born in 1986 and 1987, whose mothers during the pregnancy were living in the settlements with ¹³⁷Cs soil contamination of at least 555 kbq/m², the information was obtained from medical records on delivery and infants development. The surveyed group contained 912 cases and 1121 cases were taken as the control. The newborns with the disorders that can effect the head size, twins and the cases with incomplete information on newborns were not analyzed. The analyses were performed using parametric (t-test for independent samples) and nonparametric (Mann-Whitney U Test and Kolmogorov-Smirnov test) tests of homogeneity. The rates of infants with small anthropometrical sizes, determined from centile tables for the gestational age, were compared using ² test. To determine head circumference deficiency, the centile tables according to weight were also used. The control group showed no statistically significant differences in the length and weight of the newborns. On the contrary, the mean head circumference of newborns from the exposed group was 1.1 cm smaller than in the control. Moreover, the rate of newborns with small head circumference was twice as high in the exposed group (4.1% versus 2.0%, for their gestational age and 1.2% versus 4.4%, when weight was considered). We tend to relate the finding with radiation exposure of the growing brain of the fetus and to verify, if a deficient head circumference of newborns with other normal anthropometrical sizes is a mild microcephaly.

Unilateral Split Hand/Foot Malformation, Hypogonadotropic Hypogonadism and Anosmia: Variation of Cleft Hand-Absent Tibia, Ectrodactyly-Ectodermal Dysplasia Clefting or Kallmann Syndromes? *J.F. Macayran¹, P. Lee¹, E. Vilain^{1,2}*. 1) Pediatrics, Univ California, Los Angeles, CA; 2) Human Genetics, Univ California, Los Angeles, CA.

We describe an 18-year-old male with unilateral ectrodactyly, developmental delay, hypogonadotropic hypogonadism with bilateral cryptorchidism, hearing loss, myopia, and anosmia.

He was born with a hand deformity and leg length discrepancy. The left hand and foot were normal. The right upper extremity had a split hand malformation with three rays. The distal right tibia was hypoplastic. The right foot was adducted and small but had five toes. He also had: developmental delay, mild mental retardation, hearing loss, myopia, anosmia, micrognathia, patent ductus arteriosus, rib and vertebral anomalies, scoliosis, osteoporosis, bilateral cryptorchidism, and hypogonadotropic hypogonadism. High resolution karyotype was 46,XY.

This constellation of symptoms involving ectrodactyly of the hand and lower limb anomaly falls into the category of cleft hand and absent tibia, also known as split-hand/foot malformation with long bone deficiency, initially described by Roberts in 1967. However, there are also features that fit more in the spectrum of ectrodactyly-ectodermal dysplasia and cleft lip/palate syndrome (EEC) in which hearing loss, vision abnormalities, cryptorchidism, and hypogonadotropic hypogonadism have been described. But this case showed no evidence of ectodermal dysplasia or clefting. In addition, EEC often has bilateral ectrodactyly which is not the case here. Characteristics of anosmia and hypogonadotropic hypogonadism raise the possibility of Kallmann syndrome. Both mental retardation and hearing loss have been seen in Kallmann syndrome; however, ectrodactyly has not been described. The pituitary and olfactory defects may be attributable to a generalized clefting phenomenon occurring in EEC. We characterize this case as cleft hand and absent tibia with some features of EEC syndrome, including hypogonadotropic hypogonadism, cryptorchidism, and hearing loss.

Follow-up of an infant with harlequin ichthyosis. *L. Mehta¹, T. Cavaliere¹, P. Shineman², M. Bialer¹.* 1) Schneider Children's Hospital at North Shore, Manhasset, NY; 2) Huntington Hospital, Huntington, NY.

Harlequin ichthyosis (HI) is a rare, severe, congenital hyperkeratinization disorder. It is considered autosomal recessive. The genetic defect remains unknown. Survival is reported to be limited. A particularly distressing appearance at birth contributes to the impression that HI is lethal. Several survivors are now reported, the oldest being about 9 years. We present follow up on an infant now 2 years old. He was born to healthy non-consanguineous parents at 32 wk. gestation after premature rupture of membranes. Prior to delivery, sonogram findings included an open mouth, protruding tongue and echogenic amniotic fluid. These findings are reported elsewhere (Vohra, et al, 2003). At birth, typical findings of HI were present, with armor-like plaques of thickened, fissured skin over the body and scalp, ectropion, eclabium, small hypoplastic nose, no visible external ears, digit deformities and undescended testes. Chromosomes and TGM1 gene analysis were normal. Research studies on skin confirmed the diagnosis (Dale lab). After 6 weeks of supportive and topical care, most skin plaques had shed. Underlying skin was bright red and shiny, suggestive of ichthyosiform erythroderma. He was eating well and went home. Weight progressed at 5% and height at less than 5%. At age 2, cognitive, social and speech development appear age appropriate. He has no medical problems unrelated to HI. The most persistent plaques are on the scalp and feet. Scalp hair is present, though sparse. Teeth erupted normally. Nails are thick and require frequent cutting. Vision and hearing are normal. Ear canals require frequent clearing. Eyelids, nose and external ears remain hypoplastic. Joint limitations and thumb contractures have resulted in gross and fine motor delays. The parents of this child remain deeply committed to his care and are his main caregivers. The chief components of skin care are baths, emollients and exfoliating lotions. Despite the major psychosocial and economic impact of HI, results of treatment can be gratifying and the condition is not always lethal, as implied in the literature. This information is important for counseling parents at the time of diagnosis.

Malformations in Fetal Carbamazepine Syndrome. A. Rasalam¹, J. Reid², D. Lloyd², J. Little³, D. Shaw³, P. Turnpenny⁴, S. Moore⁵, J. Dean⁶. 1) Dept. of Child Health, University of Aberdeen; 2) Neonatal Unit, NHS Grampian; 3) Dept. of Medicine and Therapeutics, University of Aberdeen; 4) Peninsula Genetics Service, Exeter; 5) Medical Genetics Program, Memorial University, Newfoundland; 6) Dept. of Medical Genetics, NHS Grampian.

Carbamazepine is commonly used to treat epilepsy and bipolar disorder. In a population based cohort study to investigate teratogenic effects of antiepileptic drugs (AEDs) conducted in Aberdeen, Scotland, families were ascertained through maternity records indicating maternal AED use in pregnancies delivering between 1981 and 2001. 626 pregnancies exposed to AEDs were identified from a total birth cohort of 125728 (5 per 1000 livebirths). These children were born to 389 mothers, who were invited to an assessment interview. 159 mothers and their 260 children participated. Case records of those who declined, were audited, to assess any bias. In the participating cohort, 85 children were exposed antenatally to carbamazepine alone. 15 (18%) had developmental delay and 9 (11%) had major malformations. Malformations included inguinal hernia (3), submucous cleft palate (1), VSD(1), pelvi-ureteric junction obstruction (1), pyloric stenosis (2), and 2 male siblings with ambiguous genitalia (androgen receptor defect excluded). Excluding cases with hernia and pyloric stenosis, only 5 children have major malformations (6%). In the case records audit, 48 children were exposed to carbamazepine alone, of whom 4 (8%) have major malformations (2 fused labia, 1 CDH, and 1 inguinal hernia). Overall, 13/133 (9.8%) children exposed to carbamazepine alone have major malformations. In the participating cohort, a further 8 children have major malformations amongst 32 exposed to carbamazepine and other AEDs. Malformations include hernia (2), CDH(1), cleft palate (1), hypospadias (1 also exposed to valproate), neural tube defect (1), talipes equino-varus (1) and ambiguous genitalia (also exposed to lamotrigine). Genital malformations have occurred in 5 children exposed to carbamazepine alone or in combination. This association has not been reported before. We present case studies of these children to contribute to knowledge of carbamazepine teratogenesis.

Multiple congenital anomalies of unknown etiology: A retrospective review. *S.R. Sanders¹, J.A. Evans^{1,2}, A.E. Chudley^{1,2}, S. Phillips^{1,3}, B.N. Chodirker^{1,2}.* 1) Section of Genetics and Metabolism, Department of Pediatrics and Child Health; 2) Department of Biochemistry and Medical Genetics; 3) Department of Pathology, University of Manitoba, Winnipeg, Manitoba, Canada.

Accurate diagnosis of multiple anomaly syndromes is important for a number of reasons including genetic counseling and preventative healthcare. Unfortunately, the diagnosis is often not apparent at the time of the initial assessment. The diagnosis may only become available later when more syndromes are delineated or when newer technologies become available. We therefore hypothesized that a re-evaluation of previously undiagnosed cases using currently available databases would allow for a significant number of diagnoses to be made. We searched our clinic database to identify all patients with at least 2 major anomalies without a specific diagnosis. The charts including photographs were reviewed and evaluated using POSSUM, LDDDB, OMIM and Medline. An attempt was made to contact families where a new diagnostic test was available or recurrence risks were altered significantly. We identified 75 cases for evaluation. A presumptive diagnosis was suggested for 20 cases including : 22q deletion (confirmed on FISH), COFS syndrome (1 mutation identified), Smith-Magenis syndrome, Alagille syndrome, Seckel syndrome, Lambotte syndrome, Goldberg-Shprintzen syndrome, Disorganization syndrome and Holzgreve-Thomas syndrome. We therefore conclude that continued re-evaluation of undiagnosed cases with newer available technologies would often enable a diagnosis to be made.

Gene Expression Variation in Families with Hypodontia. *E. Severin, L. Boboc.* Dept Human Genetics, Carol Davila Univ Med Pharm, Bucharest, Romania.

Hypodontia is a common human anomaly affecting the tooth development of one or both dentitions. More than 200 genes are expressed during tooth development, and mutations in several of these genes cause failure of tooth formation. The isolated form of hypodontia can be sporadic or familial. The affected members within a family often exhibit variable expression of congenitally missing teeth with regard to the tooth class, region, symmetry and number of the teeth involved. Objective of the study: Variation in gene expression was suspected to produce a range of phenotypes within the families with hypodontia. *Subjects and Methods:* The study population consisted of 76 families with non-syndromic hypodontia. Six Caucasian families were selected based on the intrafamilial variability of hypodontia. The diagnosis of hypodontia has been made by clinical and radiographic examinations. The family study method was also performed. *Results:* Gene expression variation was observed in six families with hypodontia in two or three successive generations. Each affected member of the same family had different manifestations of the hypodontia. The permanent teeth most frequently missing were upper lateral incisors followed by lower second premolars and upper second premolars (excluding the third molar). Less frequently, hypodontia occurred unilateral and the left side of the upper arch was more involved. The absence of one upper lateral incisor was often associated with a small peg-shaped contralateral tooth. According to the family pedigrees the hypodontia followed an autosomal-dominant pattern of inheritance with incomplete expression of a gene defect that caused tooth agenesis and reduced penetrance on the side affected. The cases presented had no associated systemic conditions or syndromes involving both jaws. *Conclusions:* Our results provide a more direct link between gene expression variation and phenotypic outcomes. There is variable expressivity both between and within families. Individuals from different families have different mutant alleles, but individuals within the same family would be expected to have the exact same mutant allele. The mutant alleles for the hypodontia of posterior teeth are different from those of anterior teeth.

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Genetic and epidemiologic study of congenital eye malformations in 293,923 consecutive births. *C. Stoll, B. Dott, M.P. Roth.* Laboratoire de Genetique Medicale, Faculte de Medecine, Strasbourg, France.

Congenital eye malformations (CEM) were studied in 293,923 consecutive births during the period 1979-2000. The prevalence rate of CEM was 6.8 per 10,000, for microphthalmia 1.7, anophthalmia 0.23, cataract 2.7, and coloboma 1.4, respectively. Sex ratio was 0.79. Prenatal diagnosis was performed in 27 cases and 12 pregnancies were terminated. The more common types of associated malformations in the 108 affected cases (53.2%) were clubfeet, microcephaly, hydrocephaly, cleft lip/palate and facial dysmorphia. At birth infants with CEM and other malformations were smaller, weighed less and their head circumference was lower than in controls. Placental weight was also lower than in controls. Pregnancies with CEM were more often complicated by threatened abortion, oligoamnios, and polyhydramnios. Mothers of children with CEM took more often drugs during pregnancy and fathers were more often exposed to occupational hazard than parents of controls. There was a significant association between CEM and consanguinity of parents. The overall recurrence risk was 8.7%. First degree relatives of probands had more than 3 times the prevalence of non-eye malformations than controls. These results are of relevance for genetic counseling.

Prader-Willi Syndrome (PWS) with congenital hypothyroidism (CH) in a newborn with maternal uniparental disomy (MUPD15). *Y. Wang¹, J.E. Martinez¹, S.K. Bhowmick², X. He¹, C.M. Tuck-Muller¹, T.-J. Chen¹.* 1) Dept Medical.Genetics,; 2) Dept Pediatrics, Univ South Alabama, Mobile, AL 36688.

PWS is a complex genetic disorder characterized by early hypotonia, mental retardation, obesity, small hands and feet, and psychological features. The molecular basis of this disorder has been shown to be loss of genes located on paternal 15q11-13 (deletion) or their functions (MUPD15 or imprinting defect). Some of clinical symptoms suggest that PWS may be associated with hypothalamic dysfunction. However, to our knowledge, no PWS patient with CH has been reported. We report a female newborn, who presented with neonatal hypotonia and poor respiratory effort. Physical examination revealed craniofacial dysmorphism, including narrow bifrontal diameter, periorbital edema, short palpebral fissures, short upturned nose, and thin upper lip, along with low set hypoplastic nipples and long digits with flexion contractures of the PIP joints (camptodactyly). During the first week of life, low free T4 (<0.2ng/dl) and very high TSH (>340 IU/ml) led to the diagnosis of severe CH, perhaps due to dysgenetic thyroid. After thyroid hormone replacement therapy, the persistence of lethargy, universal hypotonia and feeding difficulties suggested the diagnosis of PWS. Karyotyping and FISH analysis of 15q11 were normal. Molecular studies indicated abnormal methylation on the SNRPN gene confirming the diagnosis of PWS. Linkage analysis using 13 STR markers on chromosome 15 showed that the patient inherited only one set of the maternal STR markers establishing MUPD15. The elevated TSH indicates a normal hypothalamic response to the hypo-functioning thyroid, which is not consistent with the previously reported hypothesis of hypothalamic dysfunction in PWS. It is not clear whether the CH in our patient is related to her MUPD15. The overlapping neonatal manifestations of CH and PWS (lethargy, decreased arousal, weak cry and poor suck), and the presence of unusual physical features (long digits with camptodactyly) could confuse the diagnosis and delay the identification of a newborn with PWS.

Ambiguous genitalia in a male with classical Congenital Adrenal Hyperplasia (CAH). *R. Babul-Hirji¹, D. Wherrett¹, W. Farhat¹, M. New², D. Chitayat¹.* 1) The Hospital for Sick Children and University of Toronto, Toronto, Ontario, Canada; 2) Mount Sinai School of Medicine, New York, NY.

Classical congenital adrenal hyperplasia results from an inherited enzyme defect in cortisol synthesis. More than 90% of cases are due to 21-hydroxylase (21-OH) deficiency. The androgen excess with this condition typically results in ambiguous genitalia in affected females. We report a male who presented with ambiguous genitalia and subsequently was diagnosed with 21-OH CAH.

The patient was initially seen for penoscrotal hypospadias with chordee. The phallic length was normal and the testes were palpable in the scrotum. Karyotype was 46,XY and an abdominal ultrasound was normal. At 36 hours of age, he had low LH & FSH with high testosterone and androstenedione with mildly raised 17-hydroxyprogesterone. The normal male chromosomal and gonadal sex raised the possibility of Partial Androgen Insensitivity syndrome (PAIS), although DNA analysis of the AR gene was negative. On day 13, he presented with a salt-wasting crisis with high levels of 17 hydroxyprogesterone at 231 nmol/L (normal <10). He was treated with fluodrocortisone, hydrocortisone and salt supplementation and recovered. DNA analysis of the 21-OH gene revealed an Exon 7 (V281L) mutation inherited from the father. Sequencing of the entire 21-OH gene found no additional mutations.

As ambiguous genitalia in a male is not consistent with classical 21-OH CAH, the likely explanation is this patient has two conditions: i) CAH and PAIS; ii) CAH and multifactorial inheritance of the hypospadias or (iii) CAH and a deficiency in an as yet unknown enzyme involved in the conversion of testosterone to dihydro-testosterone. The latter would result in normal external genitalia in a female with 21-OH classical CAH. This needs to be considered when counseling parents of female babies with classical 21-OH CAH born with normal external genitalia when the mothers were not on dexamethasone. This case presented a challenge not only clinically but also in genetic counseling.

Clinical Features of Familial Aortic Aneurysms and Dissections: Association with Type B Dissections, Variable Expression in Monozygotic Twins and Evidence of a De Novo Mutation Causing Disease. *D. Milewicz¹, D.*

Lemuth¹, V. Tran Fadulu¹, E. Sparks², R. He¹, B. Neichoy¹, J. Coselli³. 1) Div Med Genetics, Int Med, Univ Texas Medical School, Houston, TX; 2) Div Cardiology, Int Med, Ohio St Univ, Columbus, OH; 3) Div of Cardiothoracic Surgery, DeBakey Surg, Baylor College Med, Methodist DeBakey Hrt Ctr, Houston, TX.

Familial thoracic aortic aneurysms and dissections (TAAD) is an autosomal dominant condition with reduced penetrance and variable expression. Genes predisposing individuals to TAAD have been mapped to 5q13-14 (TAAD1), 11q23 (FAA1) and 3p24-25 (TAAD2). We studied 222 families with predominantly ascending aortic aneurysms leading to type A dissections without prophylactic surgical repair. Four families were identified with a novel clinical presentation involving both ascending (type A dissections) and descending thoracic disease (type B dissections) inherited in an autosomal dominant manner. Of the 28 affected individuals, 15 had type A disease, 4 had type B disease and 6 had both type A and type B disease. In 2 families, 3 persons had cerebral and carotid aneurysms with either ascending or descending disease. Type A dissections were preceded by aortic dilation while type B dissections were documented without aortic enlargement. 5 people had type B dissection and subsequently developed ascending aortic disease. In one family, the age at onset of ascending disease in monozygotic twins was variable, indicating the role of environmental factors in familial TAAD. Clinical history of 2 families suggest a de novo mutation, which has not been conclusively demonstrated for any other families. This study highlights the significance of paying strict attention to family history and clinical details, illuminates the importance of making an accurate diagnosis of familial TAAD in the absence of Marfanoid features, provide risk assessment in genetic counseling and medical management of this subset of families. These observations indicate the need for monitoring of the entire aorta in Type B patients. The presences of both ascending and descending disease, with cerebral and carotid aneurysms, indicate extensive vascular involvement that is a feature of TAAD.

A new observation of acro-cardio-facial syndrome points to wide interindividual clinical variability. B.

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The association of ectrodactyly, cleft lip/palate, congenital heart defect (CHD), genital anomalies and mental retardation was first reported in a Brazilian patient born to consanguineous parents. Two Italian siblings presenting with a constellation of similar defects have corroborated the existence of this syndrome, for which the acronym CCGE (Cleft palate, Cardiac defect, Genital anomalies, Ectrodactyly) was proposed. These first reports suggested an autosomal recessive pattern of inheritance. An additional newborn with ectrodactyly, cleft, ear anomalies, CHD, cortical atrophy of the brain and growth retardation has further improved the definition of this disorder which was redesignated as acro-cardio-facial syndrome (ACFS). We report on a fifth patient with ACFS born to healthy unrelated parents. The patient was hypotonic and showed facial dysmorphisms. Ears were low set, with thin upper helices, prominent and vertical antihelices, large lobes. The scrotum was hypoplastic with cryptorchidism. Right hand was split with agenesis of the third finger. Left hand was grossly normal, with a proximally implanted thumb and a relatively short first metacarpal. Lobster claw right foot was also present, with three rays which included a huge valgus hallux and abnormally modelled and partially fused fourth and fifth toes. On echocardiography a complex CHD was detected, consisting of truncus arteriosus type 1 with dysplastic and stenotic truncal valve. The patient died at one month of age from cardiocirculatory insufficiency. The present patient confirms the severe prognosis of this disorder. ACFS is a clearly recognizable disorder, manifesting with a wide range interindividual and intrafamilial variability, distinct from other known syndromes involving cleft-ectrodactyly. Lobster claw hands, which were associated with split-foot in two cases, was the only constant defect in the five known patients, while four patients showed heart defects and three patients had cleft lip/palate.

An internet-based database on the analysis of autosomal recessive congenital ichthyoses and other inherited disorders of keratinization. *C. Eckl, K.M. Eckl, H.C. Hennies.* Molecular Genetics and Gene Mapping Center, Max Delbrück Center, Berlin, Germany.

Autosomal recessive congenital ichthyosis (ARCI) is a clinically and genetically heterogeneous disorder characterized by generalized scaling of the skin and erythema. Color and shape of the scales and extent of erythema are highly variable, as well as a number of further features. A consistent genotype/phenotype correlation was not identified so far. In order to store and provide data from patients with ARCI and other related skin disorders, we have now developed a concise database service. Different phenotype variables, family history, pedigree, biochemical and histopathological data are stored for each sample. The family history will be automatically transferred to linkage compatible data formats and graphical output of the pedigree, clinical pictures will be included and presented as thumbnails. The service runs on an Apache 2 web server and is powered by a MySQL database management system and accessible through the web using an HTTP interface. For data protection reasons, sample names are stored on a separate server connected with the database by anonymous 32bit sample IDs. Data requests are handled semi-automatically via email using temporary IDs, each valid for immediate access and one dataset. The database stores genotyping data for indirect analysis of all known ARCI loci as well as mutation data obtained by direct sequencing. Further data are being collected within the German Network for Ichthyoses and Related Keratinization Disorders, which aims at comprehensively recording and characterizing such families. The service is open to external users on a collaborative basis and includes different user levels. The main purpose of the database is to assist the work of research groups by providing a common platform for data collection and exchange. Since ARCI and other keratinization disorders are rare, the database not only provides a mutation survey but is valuable for systematically collecting data. Thus, it helps to generate criteria for analyzing a potential genotype/phenotype correlation and further characterization of the phenotypic outcome of specific mutations.

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Autism work-up pitfalls; chromosome and genetic testing, metabolic disorders, seizures, immunizations, mercury, EEG, fungus, gluten and casein free diets, and brain MRI. A. Perszyk. Pediatric Multispecialty Ctr, Univ Florida, Jacksonville, FL.

Autism and related disorders are a growing diagnostic category in developmental and genetic clinics. Diagnostic criterion published (DSM IV) for this disorder is the basis for the clinical diagnosis of autism. However, incorrect application sometimes places a label that is neither warranted nor correct. Clinical features of autism are seen in a diverse group of diseases, conditions, disorders, and syndromes that initially present in childhood as the behavioral and social characteristics of infantile autism. A growing number of autism genes (20) have been mapped, both genetic and environmental factors must be considered. Purpose: To define a workable approach to identifying the etiology in children presenting with speech delay and the stereotypical behaviors of autism. Clinical work-up must include a careful search for the cause of autism when it is recognized. Method: Children that meet diagnostic criterion in the Pediatric Developmental Clinic or the Center for Autism and Related Disorders are further evaluated by Medical Genetics. Medical, social, obstetrical, and family history are obtained. Metabolic, genetic testing and imaging studies are then completed. Step-wise and individualized evaluations are completed. Conclusion: Pitfalls in the medical work-up are many and often unique to autism. Commonsense algorithm (suitable for Palm OS device) for autism work-up is presented to minimize failures, lack of follow-through, and avoidance of potholes on this diagnostic road. Obvious consequences are discussed that alter the recurrence risk estimate, dietary treatments, interventions and therapy.

The molecular basis of Ectodermal Dysplasias. *M. Priolo*¹, *M. Seri*², *C. Lagana*¹. 1) Oper. Unit of Med. Genet, Hospital Bianchi-Melacrino-Morelli, Reggio Calabria, Italy; 2) Oper. Unit of Med Genet, Pol. S.Orsola-Malpighi, Bologna, Italy.

The ectodermal dysplasias are a complex group of diseases. They all share anomalies in hair, teeth, nails and sweat gland function sorted in various combinations. The anomalies affecting the epidermal appendages are extremely variable. Few causative genes have been identified, to date. It is possible to approach this group of diseases based on functional and molecular findings and to begin to explain the complex clinical consequences of mutations affecting specific developmental pathways. By studying these pathways, we realised that all the EDs causative genes basically act through two different pathogenetic mechanisms and that clinical findings are highly peculiar for each of the two mechanisms identified, so that proposed a new classification integrating both molecular-genetic data and corresponding clinical findings. Basically, two groups have been identified. The first group includes disorders in which a defect in the epithelial-mesenchymal interaction can be recognised. All these genes regulate expression of proteins devoted to signalling between ectoderm and mesenchyme and, eventually, control differentiation and cell survival. Clinically, two key signs have to be found to fulfil inclusion criteria in this first group. Either a major ectodermal derivatives involvement as in pure EDs or a major skeletal involvement have to be present. The second group includes disorders in which an abnormal function of structural protein has been found. Causative genes present a specific pattern of expression, relative proteins being localised at the adherens junction/gap junction/ apicolateral membrane domains. Clinically, related diseases are all characterised by associated dermatologic features, mainly palmoplantar keratoderma and/or by involvement of highly differentiated epithelia. We review the molecular basis of ectodermal dysplasias in the light of this new clinical-functional classification and most recent findings in research. For each subset of ED, we describe the genes and related proteins involved in terms of: structure of the genes and their role in differentiation of the epidermis and the ectodermal derivative, genotype-phenotype correlation.

The co-occurrence of 22q11 Deletion Syndrome and Parkinson Disease: a new neurological feature? *K. Tam¹, A. Bassett^{1, 2}, E. Chow^{1, 2}*. 1) Clinical Genetics Research Program, Centre for Addiction and Mental Health, Toronto, Ontario, Canada; 2) Dept of Psychiatry, University of Toronto, Toronto, Ontario, Canada.

We recently assessed a 52-year-old Caucasian male with Parkinson Disease (PD) and 22q11 Deletion Syndrome (22qDS) in our Program. He was diagnosed with PD at 43 years of age based on prominent bilateral hand tremors. His parkinsonian symptoms have since progressed to include dyskinesia, mild dysarthria, impaired dexterity, and postural instability. He requires treatment with levodopa and ropinirole. He had a depressive episode successfully treated with medication soon after his diagnosis. He was employed, as a high school teacher but was unable to continue work approximately 2 years after the diagnosis of PD was made. The patient has not had molecular genetic testing for PD and, there was no family history of PD, movement disorders, psychotic illness, or seizures. At 44 years of age, after his son was diagnosed with 22qDS, the microdeletion was confirmed in him by standard fluorescence in-situ hybridization using the D22S75 probe. On recent physical examination, he was found to have minor scoliosis, a repaired submucous cleft palate, hypernasal speech, and minor facial features. We detected hypocalcaemia on ionized calcium testing and started him on calcium and vitamin D supplementation. It is unclear whether hypocalcaemia was of concern prior to our recent investigations because the patient had inconsistent medical follow-up for 22qDS since his diagnosis. To our knowledge, this co-occurrence of PD and 22qDS in the absence of psychotic illness has not previously been described. One previous report described the presence of parkinsonian symptoms in a patient with 22qDS and childhood-onset schizophrenia (Krahn et al., 1998). Overall, the relationship between parkinsonism in individuals with 22qDS remains unclear and, currently no overlap in the loci of these two disorders has been identified. Determining how and whether hypocalcaemia, which is common in 22qDS, may exacerbate such symptoms warrants further investigation, as does deciphering whether parkinsonian symptoms or PD represent additional characteristics in the 22qDS spectrum.

New Syndrome of Craniofacial Anomalies, Radiohumeral Synostosis, and Rhizomelic Shortness. *Z.N. Al Hassnan, A.S. Teebi.* Dept Med Genetics, King Faisal Specialist Hosp, Riyadh, Saudi Arabia .

Radiohumeral synostosis is a rare skeletal anomaly that might be seen in some craniosynostosis syndromes, notably Antley-Bixler syndrome, and in other disorders in association with skeletal anomalies. Here we report on a patient with syndromic radiohumeral synostosis in a 9 month old Saudi female of first cousin parents with unremarkable family history. Pregnancy ended at term with C-section for breech presentation. Birth weight was 2.4 kg. She was noted a peculiar craniofacial features including very large anterior fontanelle, cranium bifidum occultum, high and broad forehead, high frontal hairline, sparse scalp hair, medial thinning of the eyebrows, hypertelorism, epicanthus inversus, depressed nasal bridge, and exotropia. She has capillary hemangiomas covering the forehead, nose, and the back of the scalp. There was no proptosis and retinal exam was normal. Ears were very low-set, posteriorly angulated and malformed. Philtrum was smooth, and neck was short. Musculoskeletal examination revealed rhizomelic shortness with normal hands and feet. There was fixed flexion deformity of both elbows with no other joints deformities or contracture. There was a sacral dimple. Skeletal survey showed bilateral radiohumeral synostosis. There was no evidence of craniosynostosis on skull X-rays. Ultrasound of the kidneys revealed mild bilateral hydronephrosis. She had frontotemporal atrophy on brain MRI with no CNS malformations noted. Chromosomes were normal in blood and skin fibroblasts. She has had remarkably smooth early infantile course, continued to gain developmental milestones, though mildly delayed, and her weight and head circumference remained within normal.

The craniofacial manifestations in this patient were peculiar and, do not match any of the syndromes known to be associated with radiohumeral synostosis nor those associated with rhizomelic shortness. We think that the association is unique and apparently represents a previously unrecognized syndrome. This disorder might be autosomal recessive, though a sporadic event is a possibility.

Reliability and Validity of the Dysmorphia Checklist. *R. Blackston*^{1,2}, *C. Coles*², *J. Kable*², *R. Seitz*². 1) Div Med Gen, Dept Pediatrics, Emory Univ Sch Medicine, Atlanta, GA; 2) Fetal Alcohol Center, Marcus Institute, Emory University School of Medicine, Atlanta, GA.

We report investigations to determine if severity of dysmorphia in alcohol-exposed children is stable over time and if it is related to cognitive and behavioral outcomes. In a multidisciplinary clinic more than 500 children have been seen since 1997 and evaluated for the effects of prenatal alcohol exposure and environmental factors associated with maternal substance abuse. Characteristic physical features of FAS are measured using a 30-item Dysmorphia Checklist. To examine reliability of this assessment, records of 30 children who were seen on more than one occasion were compiled. The first assessment was done when children were a mean age of 2.9 years (SD=2.47) and the second done blindly when they were mean age 4.5 years (SD=2.52). A strong relationship between outcome of these two assessments ($r=.85$, $p<.000$) was found, indicating a high degree of consistency in the results of this exam. Differences in scores from one assessment to the next were unrelated to the length of time between assessments. The extent to which dysmorphia was related to cognitive and behavioral outcomes was examined in a sample of 275 children. We found that when children are categorized using dysmorphia scores, those with a mean dysmorphia score of 21.3 (SD=7.7) were significantly more likely ($F=4.49$, $p<.004$) to have cognitive scores in the borderline to mild mentally retarded range than were those whose dysmorphia was less severe (e.g., $M=10.4$; $SD=4.8$). Finally, in the same group, we examined behavioral ratings from the Child Behavior Checklist to evaluate whether severity of dysmorphia is related to behavior problems. We found that there were no significant relationships between physical dysmorphia and behavior problems. We conclude that the Dysmorphia Checklist is a stable, reliable tool in the recognition and diagnosis of FAS at any age. Furthermore, the degree of dysmorphia does indicate decreased cognitive levels in children with FAS but was not related to behavior problems suggesting that stable environment, early intervention services and education are important in behavioral outcomes.

A Clinical Epidemiologic Study of Patients with Split Hand Foot Malformation (SHFM) in Manitoba, Canada.

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We conducted a clinical epidemiologic population study of Manitoba to examine the incidence and epidemiology of SHFM from 1957-2003. The total number of births during this period was 842 124. Forty-five patients with SHFM were identified, resulting in an incidence of 1 in 18 713 births. The majority of patients were ascertained through referrals to the Section of Genetics at the Childrens Hospital, Winnipeg. Overall, 62% of affected individuals were females and 38% were male. The left upper limb (LUL) was the most frequently affected, with 58% of patients having an affected LUL. RUL involvement was seen in 51% of patients. Sixteen percent of patients had all four limbs affected. Split Hand Foot Malformation (SHFM) is classified as a failure of formation of parts according to the International Federation of Surgical Societies of the Hand (IFSSH) Classification and has been categorized as typical or atypical. Affected patients were classified into two main categories: Group I (typical) (31 cases) and Group II (atypical) (4 cases). However, 10 patients were not easily classified into either group and comprised a distinct category (Group III). The three groups were then subdivided into Group A (isolated SHFM) and Group B (SHFM with additional anomalies). Group IB included patients with syndromes in which SHFM has been reported (e.g. EEC, ectrodactyly with tibial agenesis and acrorenal-mandibular syndrome) in addition to patients with associated anomalies that did not constitute a well-described syndrome. Patients with isolated atypical SHFM showed preferential involvement of the upper extremities. The characteristic findings of each subgroup of patients are described and compared. The challenges involving classification, pathogenesis, etiology and implications for genetic counselling are addressed.

Goeminne syndrome-Report of a second family supporting an X-linked inheritance pattern. *B. Fernandez¹, A. MacMillan¹, B. Noble¹, M. Crowley¹, D. Jewer², S.W. Scherer³, M.O. Woods¹.* 1) Discipline of Genetics, Memorial University of Newfoundland, St John's, NL, Canada; 2) Dept of Surgery, Memorial University of Newfoundland, St John's, NL, Canada; 3) Dept of Genetics, University of Toronto, Hospital for Sick Children, Toronto, ON, Canada.

In 1968, Goeminne described a syndrome of congenital progressive torticollis, multiple keloids and cryptorchidism in an Italian family with 6 affected individuals, 4 males and 2 females. Other variable features included facial asymmetry, multiple cutaneous nevi, varicose veins and renal dysplasia. Because the females in this family were less severely affected than the males, McKusick has characterized Goeminne syndrome as X-linked, with incomplete dominance (OMIM #314300). In 1980 and 1982, two unrelated females were reported with apparently balanced X;autosome translocations and partial features of the syndrome. These women were used to map a locus to Xq28, distal to *G6PD*. Finally in 2003, a pair of Belgian authors described a 17-year old affected male, who appeared to be a sporadic case.

We report a second family, originating from Newfoundland, with this rare syndrome. The 46-year old male proband was born with torticollis, scoliosis and bilateral cryptorchidism. He is infertile and of normal intelligence. In mid-childhood, he developed spontaneous keloids and later disfiguring keloids at surgical incision sites. He has several anomalies not described in Goeminne's original pedigree including: macrocephaly, facial dysmorphism and Dupuytren's contractures. DNA on five family members was available for genetic analyses. Microsatellites markers in the region of *G6PD* on Xq28 were analyzed. A 5 Mb haplotype segregates with the Goeminne syndrome phenotype in this family. Specifically, the proband's mother carries a haplotype which was inherited by the proband and his clinically affected sister and niece. However, this haplotype was not inherited by the proband's other sister, who clinically doesn't appear to be a carrier. The haplotype includes *G6PD*, so that the disease locus may be distal to *G6PD* as previously suggested.

Trigonocephaly, short sublingual frenula, atrial septal defect, inguinal hernia, mental retardation with autosomal dominant pattern of inheritance. *M.J. Hajianpour.* Med Dir, Clinical Genetics, GENE TREK, Van Nuys, CA.

The proband is a 10 year old male with trigonocephaly, attached sublingual frenula, atrial septal defect, inguinal hernia, and mild to moderate mental retardation. His father and his half-brother through his father had reportedly inguinal hernia and short sublingual frenula. The chromosome analysis and fluorescence in situ hybridization (FISH) for velo-cardio-facial syndrome / DiGeorge syndrome (VCFS/DGS) were normal. The Opitz trigonocephaly (C syndrome) may present with the above features except for the inguinal hernia, and the inheritance of this condition is autosomal recessive. The VCFS/DGS remains as a differential diagnosis considering about 5% of individuals with clinical manifestations of VCFS/DGS may not show a deletion by FISH. Short sublingual frenula is not a reported feature of VCFS/DGS. This family may present a new autosomal dominant trigonocephaly syndrome with variable expression.

3D dense surface models identify the most discriminating facial features in dysmorphic syndromes. P.

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3D dense surface models (DSMs) can accurately discriminate controls from individuals with a dysmorphic syndrome. Here, we build separate DSMs for the peri-orbital, nasal and peri-oral areas to compare their discrimination rate to that of the full face for Noonan syndrome (NS, n=61), Smith-Magenis syndrome (SMS, n=50), Velocardiofacial syndrome (VCFS, n=95) and Williams syndrome (WS, n=65). Two pattern recognition algorithms were used to calculate classification accuracy for ten DSMs, each computed from a randomly generated 90%-10% split of the dataset into training and unseen test subsets. Below, we quote average true/false positive % rates for the most discriminating algorithm. All subjects, including over 200 controls, are Caucasian and aged less than 20 yrs, except for the SMS-control comparison where age is up to 35. For the NS-Control and WS-Control comparisons, the eyes alone (NS:93/5; WS:98/4) compare very well with the full face (NS: 97/5; WS:95/3) and are much more discriminating than either the nose (NS:73/12; WS:88/9) or mouth (NS:77/8; WS:82/4). For Smith-Magenis syndrome, the tented upper lip (80/2) always features strongly in the full face (78/4) gestalt, so the better discrimination rates of the eyes (90/4) and nose (92/5) are surprising. Given the more subtle VCFS facial phenotype, the better performance of the full face (87/5) over the nose (81/12) and eyes (81/10) was expected. The narrow mouth and shallow groove between lower lip and chin may explain the superior peri-oral performance (86/8), but exaggerated lip tension and expression in VCFS subjects cannot be discounted. Dynamic morphs between average sub-surfaces of the nose, eyes and mouth capture even subtler shape differences between control and syndrome groups than does the full face. The presentation will include comparable results for inter-syndrome comparison.

Cardiovascular malformations and deletion 3p25. A.E. Lin^{1,2}, H. Ardinger³, E. Goldmuntz⁴, T. Hoffman⁵, S. Saitta⁵, N. Spinner⁵, E. Zackai⁵. 1) Genetics & Teratology, MassGeneral Hospital for Children and; 2) Brigham-Women's Hospital (BWH), Boston, MA; 3) Prairie Village, KS; 4) Div. Cardiology, Dept. Pediatrics, and; 5) Div. Genetics, Children's Hospital of Philadelphia (CHOP), PA.

A consistent chromosomal alteration associated with specific types of cardiovascular malformations (CVMs) can lend insight into developmental mechanisms and identify candidate genes. CVMs have been reported with distal 3p del (atrioventricular canal, AVC, said to be more common) warranting a thorough review of the 3p del cardiac phenotype. PATIENTS: Of 36 literature pts with 3p25 del, and 4 new pts (1 from BWH Surveillance Program, 3 from CHOP), 36 had terminal 3p25 del, and 4 had interstitial 3q25-26 del. We excluded 16 pts with 3p25 del plus a dup or inv (9), deletion distal or proximal to 3p25 (6) and mosaic 3p25 del (1). RESULTS: Of 3p25 del pts, CVMs were present in 10/40 (25%) (all pts); and 10/32 who were informative for a CVM (31%). 3/10 (30%) had a complete AVC defect. Single pts had RtAoArch, PA/IVS, MV abn, ASD and ASD/VSD. Unspecified CHD occurred in 2. Compared to the Baltimore-Washington Infant Study [1997], AVC defects were more common (30% vs. 7%). DISCUSSION: 3p25 del is rare, and the number of pts with CVMs is small. Our review of 40 pts confirms that CVMs are common (25-30%) and that AVC defects are overrepresented. However, ascertainment bias of more severe cases is suspected. Our findings are consistent with the study of Robinson et al. [2003] that found 3 nonsyndromic pts with partial AVC had a missense mutation in *CRELD1*, a gene that maps to 3p25.3. Fernandez et al. [2004] reported a pt with 3p del phenotype but no CVM who had a bal trans with breakpoint at 3p26.2-p26.3 giving further evidence that *CRELD1* may play a critical role in the CVMs associated with 3p25 del syndrome. CONCLUSION: Del 3p25 is associated with CVMs in about 25%, 30% of whom may have an AVC defect. Pts with syndromic AVC should be carefully studied to rule out a distal 3p del. *CRELD1* and other genes mapping to 3p25 may play a critical role in causing CVMs in 3p25 del. AEL supported by CDC, Natl. Ctr. Birth Defects & Developmental Disabilities, MA. Ctr. for Birth Defects Research and Prevention, MA DPH.

Complex mosaic trisomy 7 in lymphocytes of a girl with primary microcephaly. *E. McPherson*¹, *J. Mascarello*². 1) Medical Genetics, Marshfield Clinic, Marshfield, WI; 2) Genzyme Genetics, Santa Fe, NM.

A 9-year old girl with severe primary microcephaly (-7SD) and mild developmental delay was found to have a complex mosaic karyotype 48,XXX,+7/47,XX,+mar/46,XX in peripheral lymphocytes. She was the product of an uncomplicated pregnancy to a 22 year old primigravida and her unrelated 28 year old husband. She was born at term with a weight of 3.3 kg, but was immediately noted to be microcephalic. The parents declined any genetic studies at the time. The child walked at 10 months, but her speech was mildly delayed. She spoke in short sentences at age 3. She attends school in regular classes but does require some special help in academic subjects. She has attention deficit disorder. At age 5 she developed granuloma annulare, which persists on her lower limbs. She recently required evaluation for cervical lymphadenopathy. Hematologic evaluation and lymph node biopsy were negative and the lymphadenopathy resolved spontaneously. Two subsequent siblings are clinically normal and the family history is non-contributory. Mosaic trisomy 7 has been reported in fibroblasts of several children with multiple anomalies (usually resembling VACTERL or FAVS) but was not confirmed in lymphocytes. Confined placental mosaicism for chromosome 7 has also been reported, with the fetuses usually having normal postnatal karyotype and being clinically normal or having Russell-Silver syndrome due to maternal UPD7. Clonal, but not constitutional, trisomy 7 occurs in some malignancies. Mosaic trisomy of all chromosomes including 7 has been reported in mosaic variegated aneuploidy, but despite her complex mosaicism, the proband does not meet the criteria for mosaic variegated aneuploidy. This patient is the first to be identified with mosaic trisomy 7 in lymphocytes (with the possible exception of a mother and daughter with mental illness and apparent mosaic trisomy 7 by Q-banding in a 1974 report). The marker is unidentified. The role of the marker and or mosaic triple X in the patient's phenotype (resembling microcephaly vera) and the mechanism leading to her mosaicism remain uncertain. Skin biopsy and uniparental disomy studies are pending.

A Pilot Study on the Inheritance of Pectus Excavatum. *M. Stacey*¹, *H. Harvey*¹, *V. Proud*², *R. Kelly*², *H. Creswick*², *B. Burke*², *T. Gustin*², *K. Mitchell*², *S. Blanton*³. 1) Ctr Pediatric Research, Eastern Virginia Medical Sch, Norfolk, VA; 2) Dept. Pediatrics, CHKD, Norfolk, VA; 3) Dept. Pediatrics, University of Virginia, Charlottesville, VA.

Pectus excavatum (PE) is the most common congenital deformity of the chest wall and occurs when the body of the sternum is displaced posteriorly to produce a funnel-shaped depression. The incidence of PE is approximately 1 in 1000 live births and a reported male to female ratio of 4:1. PE occurs in connective tissue disorders like Marfan syndrome (MS) and Ehlers Danlos syndrome (EDS). The etiology of PE is uncertain although familial tendency has been suggested. Studies on the inheritance of PE are limited. The purpose of this study was to determine if PE is genetic, and if so, whether it is more commonly associated in families with other connective tissue traits like lax joints. Using the EVMS Div. Pediatric Surgery database of over 1200 patients referred for evaluation of PE for possible surgical repair, we searched and identified 21 families with at least 2 affected siblings. Of these, 12 families participated in comprehensive family history interviews and pedigrees were constructed. 568 family members were collected with 281 males, 287 females and 48 individuals reported with PE including 33 males and 15 females. This was a 2.2:1 M to female ratio in this population. Statistical analyses were performed and characterized the inheritance patterns, medical problems, and associated connective tissue traits. While 3 autosomal dominant, 2 autosomal recessive, and 2 X-linked pedigrees were suggested, 5 pedigrees clearly suggested complex, multifactorial inheritance. 16 traits strongly associated with PE included long arms, legs, and fingers, high-arched palate, mitral valve prolapse, heart arrhythmia, scoliosis, lax joints, flexibility, flat feet, childhood myopia, poor healing and easy bruising were found significantly associated with PE ($p < 0.001$) even in families with no clear diagnosis of EDS or MS. Clearly, while there are single gene disorders of connective tissue that cause PE, this study suggests that it is a familial disorder associated with connective tissue and probably polygenic.

Case-control study of the muscular and skeletal geometry in neurofibromatosis type 1 using peripheral quantitative computed tomography. *D.A. Stevenson, L.J. Moyer-Mileur, J.C. Carey, S. Maxwell, J.L. Quick, C.J. Hoff, D.H. Viskochil.* Dept Pediatrics, Univ Utah, Salt Lake City, UT.

The common skeletal dysplasias of neurofibromatosis type 1 (NF1) include long-bone dysplasia, scoliosis, and sphenoid wing dysplasia. The pathogenesis is unknown. Muscle mass is important in the development of the strength of bone as voluntary muscle forces impact skeletal response and help determine the strength of bone. No studies have evaluated the bone strength and architectural structure to muscular compartments of individuals with NF1. Peripheral quantitative computed tomography (pQCT) has emerged as a modality that can address the hypothesis that NF1 individuals have decreased muscle cross-sectional area and a unique bony architecture. We examined 40 individuals with NF1 (age 5-18). Eight had a classic osseous dysplasia [sphenoid wing (1), tibial dysplasia (1), scoliosis (6)]. Cross-sectional measurements (66% tibial site) were obtained using pQCT (XCT-2000, Stratec) and variables were compared to healthy controls without NF1 (N=380) using analysis-of-covariance controlling for age, height, Tanner stage, and gender. Decreases were seen in the total cross-sectional area (NF1=6356 mm²; controls=7313 mm²; p<0.001), muscle and bone cross-sectional area (NF1=4344 mm²; controls=4986 mm²; p<0.001), muscle cross-sectional area (NF1=3886 mm²; controls=4442 mm²; p<0.001), and the Strength Strain Index (NF1=1176 mm³; controls=1314 mm³; p=0.010). When NF1 individuals were separated into 2 groups, with and without osseous dysplasias, there were statistically significant differences compared to controls, but not between NF1 groups. A trend ANOVA showed a downward trend in total [p=0.029], muscle and bone [p=0.004], and muscle cross-sectional areas (mm²) [p=0.006] between all three groups. These data suggest that NF1 individuals have decreased muscle cross-sectional area and bony strength compared to individuals without NF1. Exercise regimens may be of benefit to NF1 individuals to subsequently improve muscle and bone strength. Future studies evaluating activity levels and dietary intake may provide insights into the pathophysiology of the differences observed in this study.

A genetic and anatomic assessment of two cases of sirenomelia. *S.L. Vander Pol¹, J.J. MacKenzie², D. Situ³, G.W. Lyons³, S.C. Pang³, C.W. Reifel³, R.M.L. Smith⁴, S.A.M. Taylor¹*. 1) Pathology and Molecular Medicine; 2) Paediatrics; 3) Anatomy and Cell Biology; 4) Diagnostic Radiology, Queen's University, Kingston, ON, Canada.

Sirenomelia is a rare disorder of blastogenesis with an unknown etiology. It is characterized by partial to complete fusion of the lower limbs. We have had the opportunity to examine two term fetuses with sirenomelia preserved intact for more than 40 years at our centre. Both cases originated in the same rural Canadian community and were born within 8 months of each other. Given the geographical, temporal and superficial similarities in the physical appearances of these two fetuses we speculated that a more detailed assessment would reveal they had a similar spectrum of defects and therefore possibly a similar etiology as well. Both fetuses were subjected to an external genetic assessment, X-ray and MRI studies to identify internal and external abnormalities. FISH and molecular genetic studies were complicated by the lack of information about the original fixative used to preserve these cases. It likely had been ethanol, which leached DNA from external structures. FISH was initially used in an attempt to determine the sex of the fetuses but was unsuccessful. DNA was extracted using a guanidine isothiocyanate method from biopsies of tissue from the thymus and lung. The DNA was extremely damaged with the majority of it being degraded to less than 100 bp. The DNA was repaired by nick translation and ligation before a PCR based assay to determine their sex was performed. Our results revealed that both fetuses were male and that they had an overlapping but different spectrum of abnormalities. Principal differences were noted in ear development, brain structure, the urogenital system, the lower limbs, pelvis and vertebral column. While we hypothesize that defects of the axial mesoderm underlie the abnormalities seen in both fetuses, Fetus 1, which had only caudal defects, was found to have a spectrum of abnormalities most similar to those of the small pelvic outlet syndrome while Fetus 2, which had both caudal and cranial defects, was more similar to cases with axial mesodermal dysplasia.

Causes of death in untreated and treated Prader-Willi syndrome (PWS) individuals with growth hormone (GH).

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BACKGROUND: Obesity and short stature are among the characteristics PWS. In June 2000, the US-FDA approved GH for the treatment of growth failure in PWS children. GH has shown to have beneficial effects on growth, body composition, and respiratory function. The reports of 12 PWS children (10 M/2 F) fatalities worldwide receiving GH treatment, raised the question as to a causal connection between GH therapy and these fatalities. **OBJECTIVE:** To describe the causes of death and their relationship between weight and death in PWS individuals in comparison to the PWS fatalities receiving GH treatment. **METHOD:** A retrospective review of the circumstances of death, age and weight of 149 PWS individuals (91M/58 F) reported to the PWS Association (USA) up to June 2004. Twenty-seven subjects were under the age of 12 years, 10 between 13 and 19, and 112 between the ages of 20 and 63 years. The 12 worldwide fatalities (age 0.7 to 15.8 years), received GH for a period of 0.2 to 2 years. **RESULTS:** The cause of death was not available in 30 adults and 4 children. From 115 individuals, 42% had respiratory failure, 23% cardiac arrest, 9% sudden death, 7% choked on food, 3.5% thromboembolism, 1.7% DKA, 1.7% renal failure, and 0.9% SIDS, ovarian Ca or lung Ca. Respiratory failure as cause of death was reported in 17 (80%) and 9 (75%) in the non-GH treated and GH treated children respectively. All but two infants and one child of 149 individuals were obese. Weight was available in nine of the 12 GH treated PWS children, and all of them were obese. **CONCLUSIONS:** 1-Mortality risk is most likely increased, given the higher baseline rate of obesity and respiratory compromise in PWS. 2-Obesity and respiratory compromise as cause of death was equivalent in the GH and non-GH treated children which suggest no connection between GH and mortality risk. Larger number of children however will be necessary to compare mortality risk in GH and non-GH treated PWS. 3-Close monitoring of respiratory problems and prevention of obesity in childhood could decrease mortality risk in PWS regardless GH treatment.

Exclusion of the Two Keloid Candidate Loci 2q23 and 7p11 in a Family with Inherited Keloids and Hypertrophic Scars.

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Keloids consist of pathologic fibrosis which occurs in the skin after trauma and which grow beyond the boundaries of the injury. These cutaneous lesions are formed by excessive deposition of extracellular matrix, mainly collagen. Keloids occur in people of all racial backgrounds; however, individuals of African descent are more susceptible. Linkage of two keloid families has been reported to loci on 2q23 and 7p11 (Marneros *et al.*, 2004). We have performed linkage analysis in a Belgian family to test if it is also linked to one of these loci. The affected individuals of this family showed formation of small keloids and hypertrophic scars. Linkage analysis was performed using dense microsatellite maps inside the linked regions. The LINKAGE and GENEHUNTER packages were used for parametric (LOD) and non-parametric (NPL) analysis. They excluded both the 2q23 and the 7p11 loci. This finding provides further evidence for existence of another locus containing a gene that regulates the formation of keloids and/or hypertrophic scars. (<http://www.icp.ucl.ac.be/vikkula>) (vikkula@bchm.ucl.ac.be).

Autosomal dominant multiple familial trichoepithelioma in a large consanguineous Bedouin family: probable linkage to chromosome 16q12-13. *H. Romi*¹, *A. Zvulunov*², *R. Ofir*¹, *K. Elbedour*², *O.S. Birk*^{1,2}. 1) Laboratory of Human Molecular Genetics, Ben-Gurion University, Beer-Sheva, Israel; 2) Genetics Institute, Soroka University Medical Center, Ben-Gurion University, Beer-Sheva, Israel.

Multiple familial trichoepithelioma (MFT) is an autosomal dominant skin disease characterized by the presence of many small benign epithelial tumors with pilar differentiation predominantly on the face. The appearance of the lesions produces significant cosmetic distortion and causes much discomfort to the patients. A candidate MTF locus has been mapped to chromosome 9p21 in three north American families. Recently, mutations in the disease gene for familial cylindromatosis, the *CYLD* gene located on chromosome 16q12-13, have been shown to underlie MFT in four Chinese families. In order to identify the genetic defect causing autosomal dominant MFT in a large consanguineous Bedouin family in southern Israel, we initially performed linkage analysis with microsatellite markers from 9p21, ruling out linkage to this locus. Using 7 microsatellite markers spanning the *CYLD* gene locus at 16q12-13, we genotyped all available individuals. Our preliminary results strongly suggest linkage association to this region. Sequencing of the *CYLD* gene in the Bedouin patients is underway. These findings imply that *CYLD* defects are the cause of MFT in populations other than the Chinese.

Fetal Diagnosis of Marden-Walker Syndrome. *S.M. Nikkel¹, C. Goldsmith¹, M. Walker²*. 1) Dept Genetics, Children Hosp Eastern Ontario, Ottawa, ON, Canada; 2) Perinatology, Ottawa Hospital - General Campus, Ottawa, ON, Canada.

Marden-Walker syndrome (MWS) is a genetic syndrome characterized by blepharophimosis, joint contractures, immobile facies, decreased muscular bulk, postnatal growth retardation, developmental delay, micrognathia, and cleft palate. The fact that this syndrome has been documented in affected siblings and the children of consanguineous parents makes autosomal recessive inheritance the most likely mode of inheritance. We present a fetus who was found to have paucity of movement, bilateral talipes, and cerebellar anomalies on prenatal ultrasound. The mother was a G₂P₁ woman of Somali origin with a non-consanguineous partner. The early pregnancy was complicated by a maternal portal vein thrombosis that led to bowel necrosis. A partial bowel resection was performed at 8 weeks gestation. A detailed fetal ultrasound at 20 weeks gestation found the above mentioned features. The pregnancy was terminated and consent was given for autopsy. Karyotype was normal 46,XX. A fetal exam showed bilateral flexion deformities at the wrists. There were single palmar creases. There was minimal constriction at the elbows. Talipes was present bilaterally. The sternum was short. The face was markedly dysmorphic. Blepharophimosis and hypertelorism were present. There was a cleft palate. Internal investigations showed renal hypoplasia.

At present the molecular etiology of Marden-Walker syndrome is not known. Diagnosis is based on clinical findings. In a review paper by Williams et al. (1993), clinical criteria were devised. The majority of the mandatory criteria and supporting criteria can not be evaluated on fetal examination (developmental delay, immobile facies, and postnatal growth retardation). However very few syndromes have the constellation of findings seen in this fetus and we believe MWS to be the most likely diagnosis. MWS should be included on the differential on a prenatal ultrasound when decreased mobility, contractures and cerebellar findings are seen. Renal differences and facial changes further support the diagnosis.

Breakthroughs in molecular and cellular mechanisms underlying X-linked mental retardation. *C. Ehlers, R. Fahsold, H. Monir, K. Hager.* Institute Medical Genetics, Dresden, Germany.

Mental retardation (MR) is a frequent cause of serious handicap in children and young adults. It is defined as an overall intelligence quotient (IQ) lower than 70 associated with functional deficits in adaptive behaviour (such as daily-living skills, social skills and communication), with an onset before 18 years. Moderate to severe MR (IQ lower 50) is estimated to affect 0,4-0,8 percent; of the population and the prevalence increases to 2 percent; if mild MR (IQ between 50 and 70) is included, although these estimates vary widely between epidemiological studies. The underlying causes of MR are extremely heterogeneous. They include non-genetic factors that act parentally or during early infancy and cause brain injury, as well as established genetic causes. The Online Mendelian Inheritance in Man (OMIM) database identifies close to 1000 entries, many of which are X-linked conditions (XLMR). The prevalence of XLMR has been estimated as 1.8/1000 males with a carrier frequency of 2.4/1000 females. X-linked mental retardation (XLMR) is a heterogeneous disorder that is defined as either nonsyndromic or syndromic. To date, mutations in the following X-linked genes associated with a nonsyndromic mental retardation phenotype, although some of these genes are also associated with a specific syndromic diagnosis: *FMR2*, *GDI1*, *RSK*, *MECP2*, *SLC6A8*, *ARX*, *AGTR2* and *PQBP1*. All the genes identified to date are rare causes of XLMR-since only a small number of families have been found to carry mutations in the same X-linked gene- and there remains a large number of families with XLMR in which the causative mutation has not been identified yet.

An unusual mutational event in Duchenne muscular dystrophy: A case report. *M. Alcantara¹, M. Cervera², A. Pichardo², L. Orozco¹.* 1) Department of Human Genetics, Instituto Nacional Pediatría, Mexico city, Mexico; 2) Centro de rehabilitación Infantil Tlalnepantla, CRIT, TELETON.

ANTECEDENTS: Duchenne and Becker muscular dystrophies (DMD/BMD) are the most common heritable neuromuscular diseases in human. These diseases are inherited with a X-linked recessive pattern and are due to mutations localized in DMD gene (Xp21.2). The major types of mutations responsible for DMD/BMD are partial intragenic deletions (50-70%), followed in frequency by partial intragenic duplications (6-8%), point mutations (~30%), and other less frequent. **METHODS:** A male patient of 9 years old with diagnosis of DMD and his family, were referred to carry out molecular study and genetic counseling; no familial history of DMD was documented. The index case has a rapid progression of disease, remaining confined in wheelchair at the age of 8 years. In order to characterize the major type of mutations in the DMD gene, we carried out multiplexed polymerase chain reaction (M-PCR) of 21 exons of the DMD gene and a single PCR to analyze the neuronal promotor in the index case. DNA samples from mother and sister were analyzed by a quantitative and multiplexed PCR for evaluation of genetic dosage of deleted exons identified in the index case. As intrasample control we amplified a non-deleted exon (exon 6) and we also analyzed the DNA from healthy male and female individuals. **RESULTS:** Index case shows a double deletion pattern; one of them eliminates the neural and muscular promotors and the other eliminates exon 44, with an integrity of intermediate exons (exons 3 to 43). The evaluation of genetic dosages both in mother and sister were normal. **CONCLUSIONS:** The present case shows an unusual double deletion pattern in the DMD gene still not reported in literature. We propose that the absence of distant exons in the index case, can be explained by double de novo mutation events or by a genic inversion. The normal genetic dosage found in both mother and sister confirm the non-carrier status and support de novo mutational event.

A new microarray-based diagnostic tool for resequencing the whole CFTR gene. *M. Bonin^{1, 2}, C. May^{1, 2}, S. Poths^{1, 2}, U. Mau-Holzmann¹, D. Glaeser³, O. Riess¹.* 1) Medical Genetics Department, Institute for Human Genetics, Tuebingen, BW, Germany; 2) The Microarray Facility, Tuebingen, BW; Germany; 3) Gregor-Mendel-Laboratorium, Neu-Ulm, Germany.

Cystic fibrosis (CF) is the most common genetic disease among Caucasians. The CFTR gene consists of 27 exons and encodes for a protein that acts as an epithelial membrane channel. The recent development of a genotypic CFTR mutation screening has greatly improved diagnostic accuracy but the direct sequencing of the whole CFTR gene is expensive and time-consuming. Here we report the first microarray-based resequencing analysis of the complete CFTR gene. For this purpose, we used the oligonucleotide-microarray technology and designed a CustomSeq-Array for the coding region of the CFTR gene. The sequencing by hybridization (SBH) analysis is a promising new technology which potentially allows rapid and cost-effective screen for all possible mutations and sequence variations in genomic DNA. 35 unrelated CF patients and control persons were analyzed. All exons of each sample were amplified by PCR using specific primers, pooled, labeled, fragmented, and hybridized to the CFTR-CustomSeq array. Our approach unequivocally enables the complete resequencing of the whole CFTR gene with all exons, the exon-intron boundaries, and the corresponding promoter region with a custom-made resequencing array from Affymetrix. All examined mutations could be confirmed by the resequencing process. The tiling strategy provides additionally, in a single experiment, both complete sequencing of each strand and identification of any known or novel SNPs. The CFTR-array provides base calls at more than 99.99% accuracy which is comparable to capillary sequencing. Replicate experiments demonstrated a reproducibility of more than 99.99%. We conclude that array-based sequencing technology has the capability to efficiently and cost-effectively generate large-scale resequencing data of genes. The technology is in particular applicable to large genes with numerous different mutations, like CFTR. Furthermore CustomSeq arrays deliver a complete sequence within 48 hours which opens a revolutionary new era of sequence-based diagnostics.

Distribution of transferrin saturation and serum ferritin among Pacific Islanders living in the U.S.: the Hemochromatosis and Iron Overload Screening (HEIRS) Study. *E.L. Harris¹, B.M. Snively², T.M. Vogt¹, J.L. Holup¹, F.W. Dawkins³, V.R. Gordeuk³, H.D. Le⁴, H.V. Nguyen⁴, L.V. Passmore².* 1) Kaiser Permanente Center for Health Research (KP), Portland, OR, Honolulu, HI; 2) Wake Forest University, Winston-Salem, NC; 3) Howard University (HU), Washington DC; 4) University of California, Irvine (UCI), Irvine, CA.

Little information exists in the literature about hemochromatosis (HH) or iron overload (IO) in Pacific Islanders. The *HFE* C282Y allele is rare. In the HEIRS Study, a multi-site, ethnically diverse, primary-care-based screening study for HH and IO, we enrolled 706 participants of Pacific Island (PI) heritage. Transferrin saturation (TS) and serum ferritin (SF) levels were used to screen for IO (screening cut-offs: TS>50% and SF>300 g/L for men, TS>45% and SF>200 g/L for women). In these analyses, we included participants who did not report being previously diagnosed with HH or IO and did not have an *HFE* C282Y or H63D allele. Three of five Field Centers (KP, UCI, HU) had a sufficient number of PI participants to include in the analysis: 603 PI, 7,773 Asian, and 12,430 Caucasian participants met inclusion criteria. When adjusted for age and Field Center, PI men and women had higher mean SF levels than their Caucasian counterparts (men: 264 g/L vs 153 g/L; women: 96 g/L vs 62 g/L). Their values were similar to those of Asians (men: 265 g/L; women: 104 g/L). In contrast, mean TS levels were similar for PIs and Caucasians (men: 30.1% vs 30.1%; women: 24.2% vs 25.3%). Asians had the highest mean TS levels (men: 35.2%; women: 29.5%). The etiology and clinical significance of these differences is currently unknown. Explanations include differences among ethnic groups in iron metabolism, allele frequencies for other genes affecting iron metabolism, and the prevalence of undiagnosed primary IO, obesity, or other causes of iron elevations. Future HEIRS Study analyses include further evaluation of participants with elevated SF and TS, and gene discovery for non-*HFE* iron overload.

Sotos syndrome as a contiguous gene deletion syndrome that incorporates factor XII deficiency. *J.J. Shen¹, N. Kurotaki¹, C.W. Brown^{1,2}, J.R. Lupski^{1,2}*. 1) Dept Molecular Human Gen, Baylor Col Medicine, Houston, TX; 2) Dept Pediatrics, Texas Childrens Hospital, Houston, TX.

Sotos syndrome is a neurological disorder characterized by overgrowth, advanced bone age, dysmorphic features and mental retardation. Alterations of *NSDI* were recently proven to be a major cause of Sotos syndrome. Of a total of 145 aberrations reported, 87 represented point mutations, and deletions were found in 58 affected individuals. The frequency of microdeletion involving *NSDI* differs between Japanese and other populations; the reason for this observation is unclear. Telomeric to *NSDI* is the gene encoding factor XII (*FXII*), a serine protease in the intrinsic pathway of coagulation. Both increased and decreased levels of FXII reportedly are influenced by polymorphisms within the promoter region. The *FXII* locus lies within the common microdeletion observed in Sotos syndrome.

An individual with Sotos syndrome was determined to have persistently low levels of factor XII activity after minor head trauma resulted in a significant intracranial bleed. This combination of clinical findings was suggestive of a potential contiguous gene deletion syndrome. We conducted a cohort study in order to further test this hypothesis. FXII levels were analyzed in a total of 16 cases of Sotos syndrome with a *NSDI* deletion, and 9 control individuals. The deletion cases were 14 Japanese and two non-Japanese, and included 8 with the common microdeletion. Controls were 4 *NSDI* point mutation cases and 5 unaffected parents of deletion cases. Ten (62.5%) of the deletion patients had FXII deficiency, while in contrast, none of the control individuals exhibited low FXII levels. Based on these results, we hypothesize that the level of FXII in Sotos syndrome patients depends on the type of *NSDI* alteration. If a deletion is the causative mechanism, the remaining hemizygous *FXII* locus contributes to the total assayed level of activity, which may be in the normal range depending on the effect of promoter polymorphisms on gene expression. We propose that reduced plasma FXII levels may provide a useful surrogate marker of Sotos syndrome that arises through deletion of the critical *NSDI* region.

Autosomal recessive oculopharyngeal muscular dystrophy - an underrecognized syndrome? *B.L. Spriggs^{1, 3}, W. Ilse^{2, 4}*. 1) Molecular Diag Lab; 2) Dept of Neurology-Neurosciences, Health Sciences Ctr; 3) Dept of Biochemistry & Medical Genetics; 4) Dept of Internal Medicine, University of Manitoba, Winnipeg, Canada.

Oculopharyngeal muscular dystrophy (OPMD) is typically an autosomal dominant condition involving the trinucleotide expansion of the GCG/GCA repeat in the PABPN1 gene. Individuals with OPMD usually present in the fifth decade with progressive ptosis and dysphagia. The normal allele has 6 GCG repeats and the affected allele has 8 or more GCG/GCA repeats. The presence of two alleles each with 7 GCG repeats results in the autosomal recessive form of OPMD. There has only been one individual reported in the literature with autosomal recessive OPMD due to the presence of two 7 GCG repeats. Based on clinical findings for this one case, it was suggested that symptoms for the recessive form are milder with a later presentation in the sixth decade as compared to the dominant form. We describe a second individual diagnosed clinically with OPMD and confirmed to be homozygous for the 7 GCG repeat allele and hence, to have autosomal recessive OPMD. He presented for neurological assessment at 87 years of age with a history of ptosis, dysphagia and muscular weakness. He had no family history of similar problems and was of Anglo-Saxon descent. The dysphagia, which started 7 years earlier, had been adequately corrected by cricopharyngomyotomy 2 years prior. Examination revealed facial weakness where the patient had significant difficulties in closing his eyes and lips. There was no evidence of myotonia. The clinical findings along with myopathic EMG abnormalities lead to the diagnosis of OPMD, which was confirmed by the sizing of the GCG repeat by PCR. This represents the second known case of autosomal recessive OPMD. For this patient, the symptoms of OPMD were not noted until the eighth or ninth decade, supporting the previous thought that autosomal recessive OPMD is less severe than the dominant form. Given the reported frequency of 2% for the 7 GCG repeat allele, it is surprising that this is only the second reported case since the discovery of *PABPN1* in 1998. However, if the age of onset is frequently not until much later in life, autosomal recessive OPMD may be an underrecognized syndrome.

α^G *Xmn* I polymorphism in α -thalassemia patients in Malaysia. *Y.C. Wong*¹, *E. George*², *K.L. Tan*¹, *S.F. Yap*³, *J.A.M.A. Tan*¹. 1) Department of Molecular Medicine, Faculty of Medicine, University of Malaya, Malaysia; 2) Department of Clinical Laboratory Sciences, Faculty of Medicine and Health Sciences, University Putra Malaysia, Malaysia; 3) Department of Pathology, Faculty of Medicine, University of Malaya, Malaysia.

Beta thalassemia major and intermedia result from homozygous or compound heterozygous states for α -globin gene mutations and the clinical severity is dependent on the types of α -gene mutations involved. However, clinical severity can also be influenced by other genetic factors like increased α -globin gene expression. The *Xmn* I restriction site at -158 position of the α^G -gene has been reported to be associated with increased expression of the α^G -globin gene. The α^G *Xmn* I polymorphism causes a C to T base pair substitution at the -158 position in the promoter region of the α^G -globin gene. Increased expression of the α^G -globin gene and higher production of Hb F is associated with homozygosity for the *Xmn* I cleavage site (+/+) and thus, with less severe anemia and amelioration of the severity of α -thalassemia. Molecular characterization and frequency of the polymorphism in 107 α -thalassemia patients (58 Chinese and 49 Malays) was studied by *Xmn* I digestion after DNA amplification of a 650 bp sequence from the promoter region of the α^G -gene. Genotyping in the Chinese showed that the most frequent genotype observed was homozygosity for the absence of the *Xmn* I site (-/-) (89.7%). The 3 most common mutations in the Chinese - FS 41/42, IVS2-654 and -28 were largely associated with absence (-) of the *Xmn* I site. In the Malays, heterozygosity of the *Xmn* I site (+/-) was most common (63%). Homozygosity for the *Xmn* I site (+/+) was confirmed in 8% of the Malays and absent in the Chinese. The Chinese showed a high frequency of the (-) allele (95%) compared with the (+) allele (5%). The frequency of the (-) allele in the Malays was 60% and 40% for the (+) allele. Thus, the ratio of the (+) allele (presence of the *Xmn* I site) to the (-) allele (absence of the *Xmn* I site) was found to be higher in the Malays (0.66) compared to the Chinese (0.05).

Natural history of autosomal recessive polycystic kidney disease/congenital hepatic fibrosis. *M. Gunay-Aygun*¹, *R. Kleta*¹, *Z. Quezado*², *P. Mohan*³, *T. Heller*⁴, *P. Choyke*⁵, *W.A. Gahl*¹. 1) SHBG, MGB, NHGRI, NIH; Bethesda, MD; 2) CC, NIH; 3) CNMC; Washington DC; 4) NIDDK, NIH; 5) NCI, NIH.

Autosomal recessive polycystic kidney disease/congenital hepatic fibrosis (ARPKD) is a developmental disorder of the kidneys and liver caused by mutations in the PKHD1 gene. Fibrocystin (polyductin), the protein encoded by PKHD1, is expressed on the primary cilia of the renal tubule and bile duct epithelia and functions to maintain the tubular shapes of these structures. Kidney cysts occur as non-obstructive dilations of the collecting ducts; congenital hepatic fibrosis results from ductal plate malformation of the bile ducts. ~50% of ARPKD patients present perinatally; many die in infancy due to pulmonary insufficiency or sepsis. Both kidney and liver disease are progressive with variable rates of deterioration. More than half of the patients require renal transplant before age 20. Portal hypertension results in hypersplenism and esophageal varices. Half of the patients also exhibit cystic dilatation of the intrahepatic bile ducts (Carolis syndrome), with risk of recurrent cholangitis. Hypertension is a major problem affecting more than half of the patients. Only symptomatic therapy is available, and the natural history of ARPKD has not been comprehensively elucidated. We initiated a protocol to collect biochemical and imaging data on the hepatic and renal involvement of ARPKD. To date, 10 individuals 18 mo to 50 y have been evaluated; 4 presented prenatally. 9 had hepatomegaly and increased echogeneity of the liver on ultrasound. Magnetic resonance cholangiography showed cystic dilatation of the intrahepatic bile ducts in 3. Seven had splenomegaly and thrombocytopenia. Among the 4 who had esophagogastroduodenoscopy, 3 (ages 9, 20 and 41 years) had esophageal varices. 4 patients had subnormal renal function; creatinine clearance measurements varied from 45-110 ml/min/1.73m². Hypertension was present in 7 patients. Mutation analysis of the PKHD1 gene will allow for genotype/phenotype correlations. We continue to define the natural history of ARPKD in order to anticipate complications, provide prognoses, and identify outcome parameters for future therapeutic interventions.

Array-based CGH: identification of submicroscopic genomic disorders in clinical genetics. A. Orr-Urtreger^{1,2}, M. Goldstein^{1,2}, G. Rosner¹, A. Bar-Shira¹. 1) Genetics Inst, Tel Aviv Sourasky Medical Ctr; 2) Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

Abnormalities in DNA copy number can frequently be found in genetic disorders and tumors. This group of cytogenetic aberrations includes deletions, duplications, amplifications and unbalanced translocations. Array-based comparative genomic hybridization (CGH) allows the examination of DNA samples, derived from patients blood and tumor material, for changes in gene-copy number. By hybridizing differentially labeled test and reference genomes to arrays of hundreds genomic clones, it is possible to analyze many genes that play pivotal roles in clinical syndromes and oncology, and to detect individual and multiple changes simultaneously with high-resolution. Using the Genosensor Array 300 Chip we examined the applicability of this technique in the clinical genetic diagnostic setup. Single-copy-number gains and losses of individual genes and contiguous genes were detected in DNA samples from patients peripheral blood leukocytes. Deletions at 15q11.2-q13 in a newborn with hypotonia, cryptorchidism and hypopigmentation were detected with few discrepancies between the array results and FISH analysis. Contiguous microdeletion of *GSCL*, *HIRA* and *TBX1* genes at 22q11.2 was identified in a previously undiagnosed 12 years old boy with an unusual presentation of the VCF-DiGeorge spectrum. In an infant with multiple congenital anomalies and dysmorphic features, subtelomeric aberrations of 1qtel gain and 11qtel loss were confirmed by this method. In another case with aniridia, a borderline false negative WT1 deletion was observed, most probably because of differences between the size of the genomic deletion and the probe. Overall, good, but not perfect concordance was shown between array-based CGH and FISH diagnoses. This method is therefore becoming a useful adjunct for the rapid and accurate detection of genetic disorders associated with genomic copy number abnormalities and can significantly improve clinical genetic diagnosis and care.

LARALink: A tool for exceptional clinical utility. *J.S. Moldenhauer¹, B. Fayz², D. Wang², A. Platts², D.S. Borgaonkar³, S.A. Krawetz^{1,2,4,5}.* 1) Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI; 2) Bioinformatics Facility, Wayne State University, Detroit, MI; 3) Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, MD; 4) Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI; 5) Institute for Scientific Computing, Wayne State University, Detroit, MI.

Patient care and clinical research are somewhat limited by our ability to rapidly access the expanding pool of knowledge available in the field of genetics. LARALink (Loci Analysis for Rearrangements Link) is a web application that has been designed to allow rapid retrieval of clinically relevant information. The LARALink web application has been used as a tool for the analysis of gene expression by mining publicly available data from UniGene, dbEST, and OMIM. Recently, the site has been expanded to include a direct link to the Chromosomal Variation in Man database and the NCBI GEO database. We recently used this tool for prenatal genetic counseling of a patient with a history of a child with ring chromosome 6. Using cytogenetic parameters we were able to view a comprehensive list of the literature that is available on this condition along with highlights of the clinical features which included microcephaly, prenatal growth restriction, abnormally positioned ears, microphthalmia, hydrocephalus, and hypertelorism. This information was used to guide prenatal ultrasound to help to determine if the current pregnancy might be affected. The LARALink tool can also be applied to cases of cytogenetic aberrations, such as translocations or inversions, in attempts to make genotype/phenotype correlations through the use of cytogenetic breakpoints to identify potential gene candidates. LARALink is a tool that has tremendous potential in its applications to both clinical medicine and research. The data is expressed in a succinct and comprehensive fashion in order to allow rapid access to clinical information that helps to improve patient care and provide direction to future studies. This work was supported in part by grant 442000 to the WSU MCBI node to SAK.

Genotype-Phenotype Correlations for a Unique Disorder: Familial Myopathy, Paget Disease of Bone and Frontotemporal Dementia. *G. Watts¹, S.G. Mehta¹, J. Wymer¹, M. Pasquali², A. Kartashov³, C.D. Smith⁴, K.*

Boycott⁵, S. Markus⁶, S. Ebner⁶, S. Tucker⁷, K. Kimonis¹, A.K. Brownell⁵, V.E. Kimonis¹. 1) Dept. Genetics, Children's Hospital, Boston, MA; 2) Dept. of Pathology, School of Medicine, University of Utah, Salt Lake City, UT; 3) Clinical Research Program, Division of Biostatistics, Children's Hospital, Boston, MA; 4) Dept. of Neurology, University of Kentucky, Lexington, KY; 5) University of Calgary, Alberta Children's Hospital, Calgary, AB, Canada; 6) Praxis für medizinische Genetik, Roritzerstr. 2, 93047 Regensburg, Germany; 7) Eastover Internal Medicine, Carolinas Health Care System, Charlotte, NC.

Inclusion body myopathy (IBM) with Paget disease of the bone (PDB) and frontotemporal dementia (FTD) (IBMPFD, MIM 605382), is a progressive autosomal dominant disorder that was recently identified as being caused by mutations in the VCP (p97 or CDC48) gene. VCP is a member of the AAA protein family and has numerous functions including cell cycle control and targeted protein degradation. Within the fourteen IBMPFD families only four amino acid residues are mutated in VCP, suggesting a mutation hot-spot in the N-domain. To better characterize this disorder, we analyzed clinical, biochemical, and genetic components from 14 IBMPFD families. Haplotype analysis of the IBMPFD families identified two ancestral, disease associated haplotypes; group A: VCP mutation R155H and group B: VCP mutation R155C. The predominant IBMPFD haplotype of group A shares the same VCP mutation as four other families that do not share this haplotype. The R155C families had a significantly earlier age of onset for IBM (p0.05) compared to those with R155H. However, the R155H families had a significantly later age of onset for PDB (p0.05) when compared to R155C and the other VCP mutations. Early onset IBM or PDB did not significantly affect survival compared to the later onset of either phenotype. Interestingly we found that women were twice as likely to have dementia and APOE4 allele was associated with an increased predisposition for dementia. For the first time we demonstrate a phenotypic correlate of a given genetic change in the VCP gene in IBMPFD.

The natural history of severe anemia in cartilage hair hypoplasia. *M.S. Williams¹, R.S. Ettinger¹, P. Hermanns², B. Lee², M. Taskinen³, O. Mäkitie⁴.* 1) Dept Pediatrics, Gundersen Lutheran Medical Ctr, La Crosse, WI; 2) Department of Molecular and Human Genetics & Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas USA; 3) Pediatric Hematology, Helsinki University Hospital, Helsinki, Finland; 4) Pediatric Endocrinology, Helsinki University Hospital, Helsinki, Finland.

Case Report: A two-month old Amish infant was referred for evaluation of severe anemia. He was also found to have thrombocytosis. On examination he was noted to be short with rhizomelia and mild distal digital hypoplasia. Skeletal survey showed generalized shortening of the long bones with metaphyseal changes of the proximal femurs and femoral bowing. The diagnosis of Cartilage Hair Hypoplasia (CHH) was confirmed by homozygosity for the 70A>G nucleotide substitution in the RMRP gene. **Methods:** Additional cases of severe anemia (Hgb < 3.0 g/dl or ongoing transfusion requirement) were identified from the Finnish CHH database and from the literature. **Results:** A total of 8 patients were identified. Follow up ranged from 3 months to 40 years. All patients had severe short stature. The clinical course varied from spontaneous recovery, to ongoing transfusions and, in one case, bone marrow transplantation. Other hematologic parameters have been variable. **Discussion:** Roughly 50% of CHH patients have anemia and macrocytosis. The hematologic picture is similar to that seen in Diamond-Blackfan anemia (DBA). While the majority of patients have a mild, self-limited anemia, there exists a severe phenotype. The clinical course of this anemia is variable. The severe anemia is not due to mutational heterogeneity. The possibility of interaction between RMRP and other genes such as IGF-1 has been proposed previously. An alternative hypothesis is that changes within the protein components of the mitochondrial RNA-processing endonuclease (MRP) contribute to the phenotypic heterogeneity. It is of interest to note that 25% of DBA is caused by mutations in ribosomal protein S19 (RPS19). It is not known if RPS19 is part of the MRP complex, or interacts with the complex in some way. Genetic analysis of RPS19 is being undertaken.

Genetic heterogeneity in South African patients with the Huntington disease phenotype. A. Krause¹, C. Hetem¹, S.E. Holmes², R. Margolis². 1) Division of Human Genetics, National Health Laboratory Service and School of Pathology, University of the Witwatersrand, Johannesburg, South Africa; 2) Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, USA.

Huntington disease (HD) is an autosomal dominant neurodegenerative disorder, characterized by abnormalities of movement, emotion, and cognition and caused by a CAG repeat expansion in the huntingtin gene on chromosome 4p. A CAG/CTG repeat expansion in junctophilin-3 (JPH3) on chromosome 16q24 has been implicated in a subset of clinically diagnosed HD cases that do not have an expansion in the HD gene. The disorder caused by this mutation has been designated Huntington disease type 2 (HDL2). In a sample of unrelated South African patients tested diagnostically for HD, 84% (78/93) of white patients, but only 36% (18/50) of black patients and 50% (3/6) mixed ancestry patients had the HD mutation. However, 24% (12/50) of black patients and 50% (3/6) of mixed ancestry patients had HDL2 expansions. We have studied 17 HDL2 patients from 15 families, the largest number of families reported from any single center, accounting for 65% of the HDL2 pedigrees identified to date. Expanded repeats ranged in length from 40 triplets (the shortest known HDL2 expansion clearly associated with the disease) to 58 triplets. No juvenile cases have yet been observed, but in some families anticipation appears to be marked. Although clinical and pedigree data are limited at present, 73% (11/15) of families are clearly multiplex, with an autosomal dominant pattern of inheritance. Clinical features appear similar to HD, but remain to be fully documented. The finding that all HDL2 cases reported worldwide, including cases that we have identified in South Africa, have definite or probable African ancestry suggests a common African founder mutation; haplotype analysis is in progress to confirm this hypothesis. We conclude that HDL2 mutations account for a significant portion of the HD phenotype in South Africa, thus warranting HDL2 testing as part of the diagnostic protocol for the HD phenotype. Our findings also suggest that other causes of the HD-phenotype remain to be elucidated.

Microarray-based comparative genomic hybridization of deletion positive Angelman Syndrome subjects: Genotype-Phenotype correlations. *C.A. Bacino¹, S.U. Peters², A.L. Beaudet^{1,2}, T. Sahoo¹.* 1) Mol & Hum Gen, Baylor Col Medicine, Houston, TX; 2) Department of Pediatrics, Baylor College of Medicine, Houston, TX.

Angelman Syndrome (AS) is neurodevelopmental disorder characterized by mental retardation, dysmorphic features, ataxia, seizures and typical behavioral abnormalities including a happy sociable disposition. AS is caused by maternal deficiency of UBE3A, a gene located in an imprinted region within chromosome 15q11-13. Approximately 70 % of the AS patients have maternally derived deletions encompassing the AS/Prader Willi syndrome critical region on chromosome 15. Deletions can be divided into two groups, class I and II with 3 distinct common breakpoints (BP). Class I deletions span from BP1 to the distal most breakpoint BP3. Class II deletions span from BP2 to BP3, and since BP2 is more telomeric to BP1, class II deletions are smaller than class I. We have studied 16 deletion subjects with AS utilizing a chromosome 15 specific genomic microarray-based comparative genomic hybridization and analyzed their phenotypic severity. Array-CGH results are being confirmed by FISH studies. Those with larger deletions appeared to have a more severe phenotype as detailed below. We have also carefully characterized our cohort using standardized testing for cognitive skills, language, and adaptive behavior, and have also administered formal testing for autism: ADOS-G and ADI-R. Previously we have found that a severe phenotype is more commonly associated with a co-morbid autism diagnosis. Preliminary results from 11 of our cohort of 16 patients have been completed. Confirmatory FISH studies are underway. To date, 8/11 are class II and 3/11 class I. For class I deletions, 2 of 3 patients met criteria for autism and had more severe cognitive, language, and adaptive behavior deficits. In contrast, only 2 of 8 patients with class II deletions met criteria for autism and had more significant cognitive, language, and adaptive behavior deficits. Although those diagnosed with class I deletions appeared to be more severely affected, the sample size is being increased to determine if this is significant.

A novel mutation of CDKN1C(p57^{KIP2}) in a familial case with Beckwith-Wiedemann syndrome. *S. Ninomiya, Y. Yokoyama, M. Kawakami, H. Maruyama, T. Morishima.* Dept Pediatrics, Okayama Univ Medical Sch, Okayama, Japan.

Beckwith-Wiedemann syndrome (BWS) is a human imprinting disorder with variable phenotype. Several imprinted genes including the candidate BWS genes are clustered in chromosome 11p15.5. Recently germline mutations in the gene CDKN1C (p57^{KIP2}) have been reported in some BWS patients. We report a novel mutation of p57^{KIP2} in a familial BWS case.

Clinical features: The proband, 7-year-old girl was born at 40 weeks of gestation to nonconsanguineous parents as first-born child. The birth weight was 2,680 g. Physical examination showed macroglossia, exomphalos, ear lobe creases, cleft palate, diaphragmatic hernia, nephromegaly. She had operation for exomphalos and diaphragmatic hernia. She has no embryonal tumor. Second-born child is 2-year-old girl and is healthy. Third-born child was intra uterine fetal death at 12 weeks of gestation. Fourth-born child received the fetal ultra sound echogram examination at 20 weeks of gestation and showed exomphalos and hydronephrosis. The parents selected the induced abortion.

Cytogenetic and molecular genetic studies: The cells were prepared from peripheral blood of the parents and the proband, and from amniotic fluid of the fourth-born child. Chromosome analysis showed normal karyotype without 11p15 duplication or 11p15 translocation/inversion. The sequence of the p57^{KIP2} gene was performed by PCR product direct sequence. The overlapping fragments covered entire coding region and the exon-intron boundaries were used. In the proband the nucleotide change (codon67 CAGTAG) occurred in the exon2 and produced the nonsense mutation. The same change was detected in the mother and the fourth-born child.

Conclusions: p57^{KIP2} is a maternally expressed gene. As in this family the mutation was maternally inherited the expression of p57^{KIP2} is predicted to be inactivation in the two patients. The fact that the proband showed no overgrowth interests us the functional relationship between IGF2 and p57^{KIP2}.

Adulthood Diagnosis of Angelman Syndrome in a patient with Prader-Willi Syndrome Phenotype. *G. Scharer¹, J. Feiger², M. Taylor².* 1) Div. Clinical Genetics/Metabolism, Children's Hospital, Denver, CO; 2) Adult Medical Genetics Clinic, UCHSC, Denver, CO.

Background: Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are considered distinct genetic disorders caused by the loss of function in differentially imprinted genes on chromosome 15q11-q13. Chromosomal deletions, uniparental disomy, imprinting defects and mutations in the UBE3A gene have all been described as etiologic factors in AS, and the clinical phenotypes of both syndromes have been delineated. Recently, several cases of AS have been reported (Gillissen-Kaesbach et al., 1999) of children with a PWS-like phenotype, who were found to have methylation patterns consistent with AS. These children lack most of the typical AS features (i.e. absence of speech, ataxia, seizures, microcephaly, macrostomia or a happy demeanor), but rather present with symptoms more typical of PWS, (i.e. obesity, hypotonia and mild mental disabilities). Case Report: We now report an adult male with a history of marked mental retardation, short stature and strong food-seeking behavior, which had resulted in obesity. Except for expressive language limitations and behavioral anomalies (high level of affection), the 24 year-old patient does not display any of the classical AS symptoms. Head circumference is at the 3rd percentile, the patient has a normal gait and there is no history of seizures or ataxia. Methylation studies were performed to screen for PWS, which we suspected clinically. Southern Blot analysis and methylation sensitive PCR revealed a methylation pattern consistent with AS. High-resolution karyotyping and FISH analysis showed no evidence for structural chromosomal defects, such as deletions or duplications in the PWS-AS critical region. Conclusion: The mechanisms explaining PWS phenotypic features in patients with AS genetic lesions remain unclear. The findings in our patient indicate that AS needs to be considered in the differential diagnosis of adult patients with mental retardation and PWS-like features, in particular when food-seeking behavior and obesity are present. Methylation testing should be performed as deletion testing is likely to be normal in these patients.

Linkage Analysis and Mutation Screening in Families With Triphalangeal Thumb and Associated Hand

Malformations. *M. Sun*^{1,2}, *Q. Liu*¹, *F.X. Wu*¹, *W.H.Y. Luo*¹, *E.W. Jabs*², *X. Zhang*¹. 1) Chinese Academy of Medicine Sciences & Peking Union Medical College, Beijing, China; 2) Johns Hopkins University, Baltimore, MD.

Triphalangeal thumb (TPT) is a congenital limb malformation characterized by a long, finger-like thumb with three phalanges instead of two, and is most often transmitted as an autosomal dominant trait. TPT can occur as an isolated phenotype with variable expression and sometimes is associated with a spectrum of other limb deformities, including polydactyly and syndactyly. Triphalangeal thumb-polysyndactyly syndrome (TPTPS, MIM 190605) is an autosomal dominant genetic disorder which usually shows a duplicated triphalangeal thumb, normal index finger, and cutaneous syndactyly between fingers 3-5. The gene responsible for TPTPS was mapped to the chromosome 7q36 region in 1994 (Heutink et al.). However, the causative disease gene has not yet been identified. We have performed linkage and molecular genetic analyses in two families with TPT, a large Chinese family with typical TPTPS and a Mexican family with TPT associated with preaxial polydactyly. Linkage and haplotype analyses were carried out for 13 genetic markers covering the critical region defined by Heus et al. (1999) for the Chinese family. The data we generated confirmed the linkage of the disease phenotype to chromosome 7q36. Confirmed and predicted genes from the candidate region, *C7ORF2/LMBR1*, *HLXB9*, *RNF32*, *C7ORF13*, *LOC389602*, *LOC393076*, *LOC393889* and *LOC393890*, have been subjected to mutation screening in the Chinese family and no pathogenic mutation was identified. In both of the families, we also performed sequence analyses of the long-range SHH enhancer within intron 5 of the *C7ORF2/LMBR1* gene. A sequence alteration C/T at a conserved base of the ZRS, ZAP Regulatory Sequence domain (position 402) was found in all of the three affected individuals available from the Mexican family. This alteration was not detected in 118 chromosomes from unrelated Mexican controls by restriction enzyme Rsa I, suggesting that the alteration might be a pathogenic point mutation. [Support from the Chinese National 863 Program (2001AA221101) and the International Collaborative Genetics Research Training Program (NIH D43 TW 06176)].

WT1 mutation is a cause of Congenital Diaphragmatic hernia associated with Meacham syndrome. *W. Reardon¹, S. Smith², M. Suri³, J. Grant³, D. O'Neill³, P. Kelehan⁴, D. FitzPatrick², N. Hastie².* 1) Dept Clin Gen, Ctr Medical Gen, Our Lady's Hosp Sick Children, Dublin, Ireland; 2) MRC Human Genetics Unit, Western General Hospital, Crewe Rd, Edinburgh EH4 2XU, UK; 3) Queen's Medical Centre, Nottingham NG7 2UH, UK; 4) National Maternity Hospital, Holles St, Dublin 2, Ireland.

Disruption of the WT1 locus on chromosome 11p is known to cause human disease in Wilms tumour-Aniridia-Genital malformation Mental Retardation syndrome (WAGR), Denys-Drash syndrome and Frasier syndrome. Missense mutations within the WT1 locus have also been reported in diffuse mesangial sclerosis. The major sites of WT1 pathology thus far described in human disease relate to the kidneys and developing gonads. However expression studies during development suggest a wider role for WT1, in particular the high level of expression in the mesothelial lining of the abdominal cavity, which contribute to the developing diaphragm and epicardium. That the WT1 homozygous mouse knock-out, in addition to loss of renal and gonadal development, shows ventricular hypoplasia, pericardial bleeding and diaphragmatic hernia lends further evidence that WT1 is important in heart and diaphragmatic development. We postulate that Meacham syndrome, a rare, sporadically occurring malformation syndrome comprising congenital diaphragmatic hernia, double vagina, sex reversal and cardiac malformations might be caused by mutation of the WT1 locus. We report a missense mutation, Arg366His, in a patient with this malformation syndrome and the de novo nature of that mutation is confirmed. This is the 6th patient described with Meacham syndrome and represents the first evidence that WT1 mutation is associated with this disorder. Moreover, we present data from autopsy record review which suggest that Meacham syndrome is more common than currently recognised and we review an additional 6 cases which we have identified. We consider the implications of our observations in relation to the wider context of congenital diaphragmatic hernia and the wider appreciation of the underdiagnosed condition described by Meacham.

Familial hyper- and hypopigmentation with age-related pattern change. *J.J. Hoo, A.E. Shrimpton.* Departments of Pediatrics and Pathology, S.U.N.Y. Upstate Medical University, Syracuse, NY 13210.

An unusual genetic condition in a three generation family with cafe-au-lait and hypopigmented skin spots is reported. There are several father-to-son transmissions strongly suggesting an autosomal dominant mode of inheritance. The youngest affected family member showed one cafe-au-lait spot and one hypopigmented spot as early as the neonatal period, the oldest affected and living family member is 77 years old. During childhood, the cafe-au-lait or hypopigmented spots were circumscribed, clearly distinguishable from the surrounding normal skin, and did not follow the lines of Blaschko. However, as the affected family members grow older, both kinds of circumscribed lesions fade, and at the same time, more and more hyper- and hypopigmented freckling appears. No other symptoms of type 1 neurofibromatosis [NF1; OMIM#162200] were noted in the family. The involvement of the NF1 gene in this family was ruled out by linkage analysis using polymorphic microsatellite probes flanking the NF1 gene on chromosome 17q11. None of the affected family members showed any other feature of tuberous sclerosis [TS, OMIM #191100] or Noonan syndrome [NFNS, OMIM #601321]. Similarly there was no family history of leukemia, glioma, or hereditary non-polyposis colon cancer, which might indicate a mutation of a mismatch repair gene e.g. MSH2 or MLH1.

Germline mutations in NEP and RASA1 are associated with a subset of patients with Cowden/Bannayan-Riley-Ruvacaba syndrome. X. Zhou, R. Pilarski, K. Waite, C. Eng. Human Cancer Genetics Program, Division of Human Genetics, The Ohio State University, Columbus, OH.

PTEN is a dual specificity phosphatase and is a key regulator of the PI3K/Akt apoptotic pathway. Germline PTEN mutations, including promoter mutations and large deletions, occur in 85% of Cowden syndrome (CS) probands, 65% of those with Bannayan-Riley-Ruvacaba syndrome (BRRS) and 50% of those with Proteus-like syndrome (PSL). Germane to the clinical setting, the genetic etiology remains to be determined in a considerable proportion of CS, BRRS and PSL patients without PTEN mutation. CS is an autosomal dominant multiple hamartoma syndrome with a risk of breast and thyroid cancers, while BRRS is a congenital heritable syndrome with megencephaly, developmental delay, lipomatosis and speckled penis. Because of a variable frequency of arteriovenous malformations (AVM) component to CS, BRRS and PSL, we sought to determine if RASA1, the susceptibility gene for capillary malformation-AVM syndrome encoding RasGAP, could also be a gene for these PTEN mutation negative cases, especially those with AVM. We scanned 16 PTEN mutation negative CS, BRRS and PSL with prominent AVM for RASA1 mutations, and found one (6%) with a germline G12C mutation, not found in 100 normal control chromosomes. This individual has CS features including multiple hemangiomas, lipomas, skin tags and tonsillar and ovarian cysts. We also considered another gene, NEP on 3q25 encoding neutral endopeptidase which has been shown to recruit PTEN to the plasma membrane, enhance its stability and phosphatase activity. We scanned 62 PTEN mutation negative CS or BRRS individuals for mutations in exons 3 and 19 (encoding the steric PTEN interacting and catalytic domains). One of 54 (2%) CS and no BRRS probands had a mutation, Q22R occurring at a highly conserved residue. Further, Q22 is downstream of a series of basic amino acids that directs NEP-PTEN interaction. Thus, Q22R is predicted to disrupt this key interaction. Taken together, our data suggest that genes encoding molecules which interact with PTEN and its downstream pathways may act as susceptibility genes for PTEN mutation negative CS/BRRS.

Molecular characterization of a 230 kb candidate MR region in subtelomeric 9p. *B.L. Griggs^{1,2}, S. Ladd¹, R.A. Saul¹, B.R. DuPont^{1,2}, A.K. Srivastava^{1,2}.* 1) J. C. Self Research Institute of Human Genetics, Greenwood Genetic Center, Greenwood, SC; 2) Department of Genetics, Biochemistry & Life Science Studies, Clemson University, Clemson, SC.

Mental retardation (MR) affects 1-3% of the world wide population. Chromosomal aberrations in the subtelomeric regions have been found in 5-10% of patients with MR and developmental disabilities (MR/DD). The causative genes underlying MR/DD have not been identified in these cases. To facilitate autosomal MR gene identification, we have mapped a critical genomic region associated with MR in two unrelated patients with MR/DD and chromosomal rearrangements involving 9p24. In one patient, a male with MR, no speech, seizures, and some dysmorphic facial features (significant balding, square chin, prominent supraorbital ridges, recessed eyes, and midface hypoplasia), we identified a genomic deletion of approximately 230 kb in subtelomeric 9p. In the second patient, a female with MR and ectodermal dysplasia and a balanced translocation $t(X;9)(q13.1;p24)$, we mapped the 9p24 breakpoint to a region overlapping with the centromeric end of the 230 kb subtelomeric deletion. The ectodermal defects in the female patient are due to the disruption of the EDA gene at Xq13.1. Thus, the MR in the patient with the translocation is presumably associated with the 9p24 breakpoint. Genes located within the critical region are being analyzed to determine if haploinsufficiency or gain of function of a gene deleted or present at or near the deletion/translocation breakpoints contributes to the MR in the patient with the $t(X;9)$ translocation. The finding of a genomic interval common to two patients with MR/DD supports the notion that at least one central nervous system specific gene affecting neurological development is located within the 9p24 critical region.

Intellectual Indicators in Adults with 22qDS. *M.S. Watson¹, D.A. Young¹, A.S. Bassett^{1, 2}, E.W.C. Chow^{1, 2}.* 1) Department of Psychiatry, University of Toronto, Toronto, Canada; 2) Centre for Addiction and Mental Health, Toronto, Canada.

Background: Past research has revealed a consistent finding of higher Verbal IQ (VIQ) than Performance or non-Verbal IQ (PIQ) scores in children and adolescents with 22q11 Deletion Syndrome (22qDS). This has suggested that a non-verbal learning disability may be a common feature of 22qDS (Swillen 1999 et al., Woodin et al., 2001). However, little is known about IQ test performances in adults with 22qDS, and if this Verbal and Performance IQ split is maintained into adulthood. **Objective:** To compare verbal and performance IQ scores in adults with 22qDS. **Methods:** Full WAIS-R intelligence protocols were used to determine full-scale VIQ and PIQ scores in 47 adults with 22qDS (26 M, 21 F; mean age = 28.34 years). 25 subjects had a DSM-IV diagnosis of schizophrenia (SZ) and 22 participants had no history of psychosis (NP). **Results:** There was no significant within-subject difference between VIQ and PIQ for the whole sample (mean VIQ = 71.94, SD = 7.4, mean PIQ = 71.53, SD = 8.3, paired $t = 0.5$, $p = 0.62$). Results were similar when the subgroup of subjects with schizophrenia was analyzed separately (mean VIQ = 70.32, SD = 6.7; mean PIQ = 69.8, SD = 6.0, paired $t = 0.63$, $p = 0.54$) from the non-psychotic subgroup (mean VIQ = 73.77, SD = 7.8; mean PIQ = 73.5, SD = 10.0, paired $t = 0.18$, $p = 0.86$). There were also no significant differences between the SZ and NP groups on VIQ scores (mean difference = -3.45, SD = 7.3, $t = -1.63$, $p = 0.11$) or PIQ scores (mean difference = -3.7, SD = 8.1, $t = -1.51$, $p = 0.14$). **Conclusions:** These results indicate that adults with 22qDS do not show the reported difference between VIQ and PIQ that has been found in children. Notably, results were not affected by the presence of a psychotic illness. These results suggest that the observed VIQ - PIQ difference observed in children may be related to an uneven neuro-developmental process in 22qDS.

Gonosomal mosaicism in a family with segmental neurofibromatosis type 1 (SNF1). *C. Consoli*¹, *S. Green*², *D. Balderson*³, *D. Cooper*¹, *C. Moss*², *M. Upadhyaya*¹. 1) Institute of Medical Genetics, Cardiff University, Cardiff, Wales, UK; 2) Departments of Dermatology and Neurology, Birmingham Children's Hospital, Birmingham B4 6NH, UK; 3) Selly Oak Laboratory, Birmingham B29 6JD, UK.

Segmental neurofibromatosis type 1 (SNF1) is characterised by the regionally limited distribution of the cutaneous features of NF1. SNF1 is not infrequent (1 in 50,000) but the majority of reported cases are sporadic. The identification of NF1 gene mutations in children with classical NF1, one of whose parents has segmental NF1, should provide the means to study the development of affected and unaffected tissues in the parents. We have studied a family in which the daughter has generalised NF1, whilst her mother exhibits symptoms of segmental NF1. A nonsense mutation in exon 31 (R1947X) of the NF1 gene was identified in the lymphocyte DNA of the affected child by denaturing high performance liquid chromatography (dHPLC). Skin biopsies were taken from both affected and unaffected regions of the mother's body; fibroblasts and keratinocytes were then cultured. Direct mutation analysis of NF1 exon 31 failed to identify the R1947X mutation in DNA samples derived from these cell lines. DNA fragments containing exon 31 were then cloned from each cell line and clones were screened using allele-specific PCR. The R1947X mutation was identified in 19% (29/146) of keratinocyte clones derived from the mother's affected tissues and in 11% (12/136) of clones derived from affected fibroblasts. The nonsense mutation was however undetectable in clones derived from clinically unaffected tissues. This finding provides support for the postulate that some patients with segmental neurofibromatosis are gonosomal mosaics. Thus, the mother with segmental NF1 is presumed to be a gonosomal mosaic who expresses the mutant NF1 allele both in her germline cells and in a variable proportion of her somatic cells. These data should potentiate both molecular diagnostic testing and the provision of genetic counselling in family numbers, but could also lead to a better understanding of the molecular mechanisms underlying SNF1, by helping to identify the cellular lineages manifesting the mutation.

Hutterite brothers both affected with 2 forms of limb girdle muscular dystrophy (LGMD2H and LGMD2I). P. Frosk, K. Wrogemann, C.R. Greenberg. University of Manitoba, Winnipeg, MB, Canada.

Limb girdle muscular dystrophy is very common in the Hutterite population of North America. We have recently reported the homozygous D487N mutation in *TRIM32* associated with the mild form of Limb Girdle Muscular Dystrophy type 2H (LGMD2H). We have also identified Hutterite patients with LGMD2I, homozygous for the common L276I mutation in *FKRP*. To date all Hutterites with LGMD have been shown to have one of these two mutations. A comparison of the 2 phenotypes in the Hutterites is in press (Human Mutation). We now report a Hutterite family ascertained by a father known to have LGMD2H. Two of five sons (7 and 10 years old), studied due to parental concern of decreased stamina, were found to be homozygous for **both** the *TRIM32* D487N and *FKRP* L276I mutations. Both had slender builds with no muscle hypertrophy or atrophy, no objective signs of muscle weakness, normal cardiac and respiratory exams, and elevated CK values of 1555 and 1055 U/L respectively (normal <165 U/L). The older boy had a muscle biopsy showing an active dystrophic process. Echocardiograms are pending. The other 3 sons (4, 6 and 11 years old), currently asymptomatic, had normal physical examinations and CK levels of 160, 151 and 213 U/L respectively (normal <165 U/L). They were found to be homozygous and heterozygous for the *TRIM32* and *FKRP* mutations respectively, identical to their asymptomatic 33 year old mother (CK level 117 U/L; normal 28-110 U/L) and their 40 year old affected father who had moderate slowly progressive proximal muscle weakness and a CK level of 2030 U/L (normal 52-175 U/L). Our experience with 45 Hutterites affected with LGMD2H alone and 21 Hutterites with only LGMD2I suggests that LGMD2I is more severe with an earlier age at onset and higher CK levels. Preliminary analysis of these 2 boys with both forms of LGMD does not reveal differences in age at or mode of presentation, physical findings, or CK levels compared to individuals affected with LGMD2I alone. This suggests that the effects of these two mutations are not acting synergistically at a young age. It remains to be seen whether there will be signs of interaction between these two LGMDs as the patients get older.

Description of a New French-Canadian Recessive Form of Limb-Girdle Muscular Dystrophy with Prominent Quadriceps Atrophy. *J. Jarry¹, M.F. Rioux¹, M. Vanasse², Y. Robitaille², G. Karpati³, B. Brais¹.* 1) Laboratoire de Neurogenetique, Centre de Recherche du Centre Hospitalier de l'Universite de Montreal, Quebec, Canada; 2) Hopital Ste-Justine, Montreal, Quebec, Canada; 3) Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada.

Limb-girdle muscular dystrophies (LGMDs) are a heterogeneous group of disorders characterized by progressive weaknesses of the limb girdle muscles. Advances in genetics have allowed the characterization of ten recessive forms. We have identified a cohort of sixteen individuals from twelve different French-Canadian families displaying a phenotypically distinct recessive form of LGMD associated with quadriceps atrophy and myalgia. All patients were examined, genealogical data was collected, serum creatine kinase (CK) levels were measured in blood samples from affected and non-affected individuals alike, and EMG results and muscle biopsies were reviewed. Males and females are equally affected, while the number of affected cases is consistent with a recessive mode of inheritance. CK levels are always elevated but demonstrate important variability even in the same family. (from 1.5 to 30 times normal) EMG results show mostly myopathic changes. Immunohistochemistry studies of muscle biopsies demonstrated normal staining for dystrophin and sarcoglycans. Using an affected-only homozygosity mapping strategy on two of the largest families we performed a 10-cM genome-wide scan using the Weber 8 panels. Analysis of the different candidate regions is on-going. This study is the first description of a large French-Canadian cluster of cases of a new recessive LGMD with quadriceps atrophy.

An alternative for FISH: MLPA-based detection of genetic imbalances (aneuploidies), from sample to result within 6 hours. *M.E. Kalf^{1,2}, S.J. White¹, M. Kriek¹, L. Vahlkamp², R. van Beuningen², J.T. den Dunnen¹, G.J. van Ommen¹, M.H. Breuning¹.* 1) Leiden University Medical Center, Leiden, Netherlands; 2) PamGene International B.V., 's-Hertogenbosch, Netherlands.

Diagnosis of syndromes caused by chromosomal abnormalities is performed using cytogenetic analysis. Since cells need to be cultured, a diagnosis takes at least 3 days. In some cases, for example trisomy 18 and trisomy 13 the prognosis is such that treatment is usually withheld. The time required to determine whether these chromosomal aberrations might be involved or not is an important issue. If not, the appropriate treatment can be arranged in a timely manner.

We have tested Multiplex Ligation-dependent Probe Amplification (MLPA) in combination with a porous micro array substrate (PamChipTM array) as a tool for detection of such aneuploidies. Compared with planar arrays, the PamChip array has several advantages, including the larger surface area, the possibility to vary hybridization stringency during analysis and most interestingly a decreased hybridization time of about 10 minutes.

For each of the chromosomes to be tested (13, 18, 21, X) six probes were designed, as well as some control probes. To facilitate array-based analysis, each MLPA probe contains a non-hybridizing tag sequence. For analysis, oligonucleotides complementary to the tag sequences were spotted on the PamChip.

By comparing spot intensities between controls and patients it was possible to detect different aneuploidies within 6 hours from blood sample until result. Using the methodology developed, we performed a blind study on 25 patients and typed all correctly. Preliminary results showed that even mosaic cases (50%) could be detected. To facilitate wide-spread application several protocols were tested successfully, including MLPA performed on buccal swabs, amniotic fluid samples and amplified genomic DNA. The limit of the method was tested using 200 probes in one assay; excellent results were obtained. Our results thus indicate that it should be feasible to design powerful assay to screen for genetic imbalances with a resolution at the gene level.

Cutis laxa arising from an autosomal dominant splice mutation in exon 24 of the elastin gene (ELN) in a three months old boy and his father with highly variable phenotype. *C. Kraus¹, I. Hausser², M. Essayi³, J. Kunze⁴, A. Reis¹, L. Neumann⁴.* 1) Inst Human Genetics, Univ Erlangen, Erlangen, Germany; 2) Electron Microscopic Laboratory, Dermatology, University Clinic, Heidelberg, Germany; 3) Department of Neonatology Charit Campus Mitte, Berlin, Germany; 4) Institute of Human Genetics, Charit Campus Virchow-Klinikum, Berlin, Germany.

Congenital cutis laxa is a rare, genetically heterogeneous connective tissue disorder. The systemic involvement of elastic tissue is characterized by high clinical variability. The typical aspect is marked skin laxity lacking elastic recoil which is accompanied nearly always by rarefaction, loss, fragmentation or severe disorganisation of dermal elastic fibres. In three cases of autosomal dominant cutis laxa, causal frameshift mutations within the gene coding the structural protein Elastin, a main component of elastic fibres, could be identified. Here we report a three month old boy with cutis laxa and his clinically unaffected father. Mutation analysis in the Elastin gene was carried out by sequencing all coding exons in genomic DNA. A cytosine to thymine substitution was identified at the last position of exon 24. To further characterize the implication of the mutation, total RNA was extracted from cultured fibroblasts. RT-PCR and subsequent sequencing of a fragment encompassing the critical region revealed an in frame deletion of exon 24, probably, resulting in a stable protein lacking amino acids 526-540. This is consistent with former observations that suggest that the phenotype of cutis laxa is the result of a dominant negative effect compared to SVAS caused by loss of function mutations in the ELN gene. Interestingly, the mutation was also present in the father who had no signs of cutis laxa, suggesting that the mutation is associated with a high phenotypic variability. Nevertheless, herewith we report the fourth family with autosomal dominant cutis laxa, caused by a mutation in the Elastin gene.

Construction of high throughput gene analysis system using DNA microarrays. *Y. Takahashi, J. Goto, S. Tsuji.*
Neurology, The University of Tokyo, Bunkyo-ku, Tokyo, Japan.

[Introduction] With recent progresses in molecular genetics, a number of causative genes for hereditary diseases have been identified, and it is imperative to identify individual mutations of the corresponding genes in clinical practice. Given this background, we are confronted with strong demand for a high throughput system capable of analyzing individual genes from a large number of patients. To accomplish this aim, we have designed new microarrays using GeneChip™ CustomSeq resequencing system (Affymetrix). We designed three microarrays: TKYPD01 for Parkinson disease and related disorders, TKYALS01 for amyotrophic lateral sclerosis (ALS), and TKYAD01 for Alzheimer disease and other dementias. These microarrays contained the exon and their flanking intron sequences of the causative genes, disease susceptibility genes, modifier genes, and related genes which presumably play a key role in these diseases. Promoter sequences of several genes were also included. The purpose of this study is to validate the accuracy of these microarrays to identify individual mutations. [Materials and Methods] Genomic DNA samples were obtained from patients with familial ALS as well as from normal controls with informed consent. Twelve DNA samples with seven SOD1 mutations (L129*del(TT), G93S, I149T, V118L, H46R, L84V, and S134A) and a polymorphism, all of which had been previously identified using direct nucleotide sequencing, were used. The genomic DNA was amplified using specific primers for each exon, and the PCR products were pooled, fragmented, labeled, and hybridized on TKYALS01 microarrays. [Results and discussions] All the point mutations and the polymorphism were unambiguously detected. For the deletion mutation, the signals of probes at the deletion site, as well as those of ten adjacent probes, were apparently weak compared with those derived from normal controls. In addition, we found previously unidentified single nucleotide polymorphism at 5-UTR in two patients. These results indicated that this microarray system was a robust tool for identifying point mutations, and potentially useful for deletion mutations as a high throughput analysis system.

Pseudoxanthoma Elasticum: mutation analysis and phenotypical characterization in Belgian patients and heterozygous carriers. *O. Vanakker^{1,3}, P. Coucke¹, B.P. Leroy¹, D. Voet², E. De Baere¹, P. Van Acker¹, A. De Paepe¹.*
1) Center for Medical Genetics, Ghent University Hospital, Belgium; 2) Department of Internal Medicine, Ghent University Hospital, Belgium; 3) Research Assistant of the Fund for Scientific Research Flanders (Belgium).

Pseudoxanthoma Elasticum (PXE) is a heritable connective tissue disease comprising cutaneous, ocular and cardiovascular manifestations. It is caused by mutations in the ABCC6 gene on chromosome 16p13.1. It is generally believed that the inheritance of PXE happens in a recessive manner. Systematic clinical and molecular screening of carriers has, to our knowledge, not yet been reported.

We present 38 Belgian families of PXE-patients. The diagnosis was established on clinical and histological grounds. Molecular analysis revealed 63 mutations, 15 of which are novel. Different types of mutations were found in homozygous (22%), heterozygous (25%) or compound heterozygous (53%) form, although the R1141X mutation is most frequent.

In a subgroup we analysed the phenotype of patients and carriers through history-taking, clinical, ophthalmological and ultrasonographical examinations. We found the typical skin and retinal lesions in all patients but in none of the carriers. In 45% of PXE patients we detected calcifications in the liver, kidneys and spleen. Remarkably, some of the carriers showed similar lesions. In addition we found bilateral microlithiasis in all male patients so far but also bilateral calcification of the rete testis in carriers. These findings suggest that in both patients and heterozygous carriers, calcifications of the abdominal organs and testicles may be part of the PXE phenotype. Since the latter are considered a risk factor for malignancy, follow-up is warranted.

In patients homozygous for the R1141X mutation, we looked for genotype-phenotype correlations. Although this needs further confirmation, this subgroup seems not only to have a more severely affected cardiovascular system, but also severe retinal problems. Further correlations are still under investigation.

Body Temperature and Sleep Disturbance in Smith-Magenis Syndrome (SMS). *W.C. Duncan¹, R.S. Morse², D. Krasnewich¹, A.C.M. Smith¹.* 1) MAP/NIMH; 2) MGB/NHGRI, NIH/HHS, Bethesda, MD.

The sleep disturbance in Smith-Magenis syndrome (SMS) extends from early childhood through adulthood and is the major correlate of negative behavior during childhood. The disturbance is characterized by increased daytime sleepiness and reduced nighttime sleep that is strongly linked to early morning awakening. The disturbance may be partly attributed to an inverted 24-hour pattern of melatonin (low nighttime levels and elevated daytime levels). In all other organisms, melatonin is elevated at night. In addition, the sleep disturbance could be related to an altered timing of the circadian rhythm in body temperature. In humans, the 24-hour rhythms of melatonin and body temperature are often used as biological markers of the central circadian clock. Therefore, the unique phenotypic inverted melatonin pattern observed in SMS suggests that circadian clock phase is inverted in SMS. Preliminary evidence argues against this, since the timing of the circadian rhythms of cortisol and growth hormone are relatively normal in SMS. To more definitively estimate circadian clock phase and its possible relationship to disturbed sleep in SMS, the circadian rhythm of body temperature was measured in 6 subjects with FISH confirmed SMS (age 4-13 y). Temperature was measured at home from the ear or forehead once every 3 hours for 48-72 hours by parents who were trained in the procedure. Care was taken to measure temperature only during quiet behavior and rest. For each subject the circadian rhythm was formally characterized by a least-squares sine wave fit to the data. In each case a clear non-inverted circadian rhythm of body temperature was identified with circadian peaks present during the daytime and troughs at night. The data suggest that the circadian clock is not inverted in SMS. Further, the fact that the average temperature peak tended to occur early in SMS (12:45PM 2:20h:m) suggests that early timing may be related to the early morning awakening commonly seen in SMS sleep patterns. If so, such information would be beneficial for understanding the specific biological basis of the sleep disturbance, and for developing possible treatment strategies.

The repetitive behavior subphenotype in autism. *M.L. Cuccaro¹, S.L. Donnelly¹, C.M. Wolpert¹, R.K. Abramson², H.H. Wright², G.R. DeLong¹, A. Ashley-Koch¹, M.A. Pericak-Vance¹.* 1) Center for Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) WS Hall Psychiat Instit, Univ of South Carolina, Columbia, SC.

Restricted and repetitive behaviors and interests are a defining feature of autism. A repetitive behavior subphenotype has been developed to create subgroups for genetic analyses of autism. The current study examined developmental influences (chronological age and developmental level) on the repetitive behavior subphenotype. 162 participants with autism (age range 3 to 21 years) were drawn from an ongoing autism genetics study. Participants consisted of 130 males (M age = 94 months, SD = 45) and 32 females (M age = 105 months, SD = 44) from both family history positive and negative families. Clinical diagnostic and behavioral assessments included the Autism Diagnostic Interview-Revised (ADI-R), Aberrant Behavior Checklist (ABC), and Vineland Adaptive Behavior Scales (VABS). The repetitive behavior subphenotype was measured using the ADI-R and The ABC. Using the ADI-R, two repetitive behavior factors were constructed: Repetitive Sensory Motor Behavior (RSMB) and Insistence on Sameness (IS). The ABC Stereotypy Factor was also examined. Analyses indicated that the RSMB factor was significantly negatively correlated with both age ($r = -.16$, $p = 0.04$) and developmental level ($r = -.20$, $p = 0.01$). The IS factor was significantly positively correlated with age ($r = .16$, $p = 0.05$). While significant, these correlations suggest weak relationships. The ABC Stereotypy factor score failed to attain significant correlations with either age or developmental level. Multiple regression analyses were conducted using age, developmental level, and RB measures as predictors. For the RSMB and IS factors, predictor variables account for a limited amount of the variance (RSMB $R^2 = 14\%$; IS $R^2 = 11\%$). Both age at exam and the VABS Adaptive Behavior Composite score contributed significantly to the dependent variables. The results suggest that the repetitive behavior subphenotype is not uni-dimensional. Given the increasing use of this subphenotype in genetic analyses of autism, it is critical that subsequent investigations explore the full range of this potential subphenotype.

Unusal phenotype for cystic fibrosis. *W. Eyaid*¹, *M. AlBalwi*², *M. Al-Ghahab*³. 1) W.Eyaid, Dept Pediatrics, MBC 1510, King Abdulaziz Medical City, Saudi Arabia; 2) M. A. AlBalwi, Departemnt of Molecular Pathology and Genetics King Abdulaziz Medical City, Riyadh, Saudi Arabia; 3) M. Al-Ghahab, Dept of Pediatrics, MBC 1510, King Abdulaziz Medical City, Saudi Arabia.

A 14 months old boy was born for a first cousin parents as a product of 40 wk gestation with appropriate birth growth parameters. The parents lost a daughter at 7/12 of age with a history of failure to thrive (FTT). She had normocytic normchromic anemia, hepatosplenomegaly, hypoalbuminemia and congenital amputation of right hand. Our patient was admitted at 2 months of age to investigate his anemia, thrombocytopenia and FTT. On examination he had postnatal growth retardation, fair skin, blond spare scalp hair, hepatosplenomegaly and petechial rash. His Hemoglobin electrophoresis was normal and ranging between 6.5-7.2. Serum ferritin was 1151 and normal serum copper and ceruloplasmin were seen. Normal Bone marrow aspiration and liver biopsy showed iron deposit. Work up for storage disease like Nieman-pick and Gaucher was unremarkable. He developed chronic cough and readmitted for bronchiolitis and diarrhea. Elevated sweat chloride test was seen three times at age of 7/12 and result showed 111 mmol/L, 95 mmol/L and 107 mmol/L (NL < 60 mmol/L) respectively. He was started on medication for chest and diarrhea. On follow up, his anemia resolved and his liver was just palpable. His height and weight were 50% for 7 months at 14 months of age. DNA mutation analysis for cystic fibrosis showed presence of 1548delG mutation in exon 10 in both alleles of the CFTR gene. This is a common pathogenic mutation reported among Saudi population. Our cases showed a significant difference in the CF clinical presentation that have not been reported before, however, they had the common mutation that may have some influence on clinical presentation due to CFTR gene modification product.

Phenotypic Heterogeneity in Congenital Cataract Cases from India. *V. Kumar¹, D. Singh², K. Guleria¹, K. Sperling³, J.R. Singh¹.* 1) Centre for Genetic Disorders, Guru Nanak Dev University, Amritsar, Punjab, India; 2) Dr. Daljit Singh Eye Hospital, Sheranwala Gate, Punjab, India; 3) Institute of Human Genetics, Augustenburger Platz 1, 13353, Berlin, Germany.

Methods: More than 710 cases affected with bilateral congenital cataract were investigated at Dr. Daljit Singh Eye Hospital, Amritsar. Detailed ophthalmic examination, that included slit-lamp examination and 3-D photography of the lens to record the cataract type, was undertaken in all the cases. **Results:** Apart from the few known cataract types like; polar, nuclear, total, zonular, sutural, cerulean, coralliform etc. we have reported more than 100 distinct phenotypes in these cases, over 20 being novel phenotypes, not documented in literature so far. **Conclusions:** We have observed a wide phenotypic heterogeneity in our cases of congenital cataract, indicating its inherent genetic heterogeneity. The identification of responsible genes, causative mutations and modifiers will be of great help to draw a precise correlation between genotypes and different phenotypes observed so as to understand the exact mechanism of cataractogenesis, but the utmost need is of a uniform classification for congenital cataract.

Hair whorls and Handedness: Informative phenotypic markers in non syndromic cleft lip with or without cleft palate (NS CL/P) cases and their unaffected relatives. *N.M. Scott, S.M. Weinberg, K. Neiswanger, M.L. Marazita.*
Center for Craniofacial and Dental Genetics, University of Pittsburgh, Pittsburgh, PA.

CL/P is a common birth defect with a wide range of birth prevalences (1/500-1/2000) and a complex etiology. Studies have demonstrated differences in brain structure and cognitive traits between individuals with CL/P and controls. Accordingly, in the current study, we hypothesized that altered brain development is part of the phenotypic spectrum of NS CL/P by assessing non-right handedness (NRH) and atypical hair whorls (HW) in CL/P families. Though previous investigators have shown increased levels of NRH in CL/P, atypical hair whorls (counter-clockwise and double) are more direct markers of altered brain development, and have yet to be studied in CL/P. The study population consisted of 41 NS CL/P cases and 124 of their unaffected relatives. The Edinburgh handedness inventory was used to determine handedness; scores of 50 or less were considered NRH.

Trait	CL/P	Unaff. Relatives	All Subjects	General Pop.
NRH	31.7%	18.5%	21.7%	10.0%
Atypical HW	4.9%	21.8%	17.6%	9.9%

The proportion of NRH was increased in both the CL/P ($p < 0.0001$) and unaffected relatives ($p < 0.001$) versus the population rate, and in CL/P cases versus the relatives (though not significant). All subjects had increased rates of atypical hair whorls versus the population rate ($p < 0.001$). These results imply that aspects of brain development comprise part of the extended phenotype of orofacial clefting, both in affected individuals and their non-cleft relatives. Funded by NIH grants DE-13076, DE-016148.

Autosomal recessive distal spinal muscular atrophy (Chronic DSMA) with congenital onset : clinical picture and genetic study of 4 cases. *L. Viollet^{1,2}, I. Maystadt³, M. Zarhrate¹, A. Munnich¹.* 1) INSERM U393, Hopital Necker Enfants Malades, 149 rue de Sevres, 75743 PARIS, Cedex 15, France; 2) Service de Pediatrie et Reeducation Neuro-respiratoire, Hopital Raymond Poincare, 92380 Garches, France; 3) Department of Human Genetics, Catholic University of Louvain, Brussel, Belgium. FNRS, Brussel, Belgium.

Autosomal recessive Chronic Distal Spinal Muscular Atrophy (Chronic DSMA or Distal Hereditary Motor Neuronopathy type III) is a rare neuromuscular disorder characterized by progressive anterior horn cell degeneration, leading to motor weakness and muscular atrophy predominating in the distal part of the limbs and accounts for less than 10% of all spinal muscular atrophy cases. We collected a series of 12 European unrelated Chronic DSMA patients, with a broad range of age at onset, extending from 0 to 12 years. We mapped Chronic DSMA gene in a short genetic interval on chromosome 11q13.3. We showed evidence of a common haplotype at this locus in 8/12 patients, suggesting a partial linkage disequilibrium in the European population. Four patients presenting a Congenital Onset Chronic DSMA had a generalized hypotonia at birth with bilateral equinus varus feet and flexed fingers attitude. No neonatal respiratory distress was present. Outcome was marked by delayed motor milestones and a low progression of paralyzes during childhood. Progressive restrictive respiratory insufficiency was observed, due to diaphragmatic paralysis. Neurophysiological assays showed a severe muscle denervation with normal nerve conduction velocities and distal sensory potentials were detected. No SMN gene deletion was found in these patients. Haplotype analysis at the 11q13.3 locus revealed that all of the four patients harbored the common "European" haplotype. These data strongly supported the view that this congenital phenotype was linked to the Chronic DSMA locus on chromosome 11q13.3. Identification of the mutation responsible for Chronic DSMA will hopefully allow the molecular diagnosis of this congenital onset condition and will probably help understanding the mechanisms of anterior horn cell degeneration in childhood onset spinal muscular atrophies.

The polymorphism A1298C in the MTHFR gene is distributed according to gender, and it is associated maternally to neural tube defects in the State of Yucatan, Mexico. *L. Gonzalez-Herrera¹, I. Castillo-Zapata¹, G. Garcia-Escalante¹, D. Pinto-Escalante¹, A. Gonzalez-Del Angel².* 1) Genetics Lab, CIR, Univ Autonoma de Yucatan, Merida, Yucatan, Mexico; 2) Department of Research in Human Genetics. Pediatrics National institute.

Neural tube defects (NTD) are highly prevalent in the State of Yucatan, Mexico. NTD include congenital malformations such as anencephaly, spina bifida and encephalocele. MTHFR is a candidate gene for NTD in the folate metabolism pathway. The polymorphism A1298C in the MTHFR gene has been associated with NTD. Research has spurred interest in maternal genetic effects, because folate variants could influence the risk of NTD through the maternal, the embryonic, or both genotypes. In this study, we evaluated the frequency of the allele A1298C-MTHFR according to gender, and its association with NTD in the State of Yucatan, Mexico. We genotyped by PCR-RFLPs, 99 subjects with NTD, 82 of their respective mothers and 44 of their fathers to compare with 110 control subjects: 63 women and 47 men with healthy offspring. Genotypic frequencies in control group were in agreement with Hardy-Weinberg expectations ($p > 0.33$). The frequencies of the allele C and heterozygous AC genotype were significantly higher in males than in females ($p = 0.017$ and $p = 0.032$, respectively), suggesting an heterozygous advantage. Interestingly, we found significant association for allele 1298C ($p = 0.025$) and for the heterozygous AC genotype ($p = 0.034$) only in mothers of NTD. Comparison on the basis of the type of NTD, showed that the polymorphism A1298C was significantly associated with mothers of anencephaly cases only ($p = 0.029$). We found no evidence of a contribution for NTD through the interaction of both maternal and embryonic genotypes ($p > 0.05$). Our results suggested that the polymorphism A1298C-MTHFR is associated with the risk for NTD mainly through the maternal genotype rather than through the embryonic genotype. So, the allele C and the heterozygous AC genotypes are maternal risk factors for having offspring with anencephaly in the population of Yucatan (OR= 2.23 IC:1.12-4.46 and OR= 2.57 IC:1.14-5.78, respectively).

A genetic study on dominantly inherited spontaneous pneumothorax caused by bullous lung lesions. K.

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Primary spontaneous pneumothorax (PSP) is a fairly common condition occurring with the minimum frequency of 7,4:100 000 in males and 1,2:100 000 in females. PSP is mostly sporadic but also known to occur in families. The purpose of this study was to recognize possible lung lesions underlying inherited susceptibility to pneumothorax and to study the genetics of the condition. A large Finnish family with inherited spontaneous pneumothorax occurring in three consecutive generations was identified. Altogether 29 family members (7 with pneumothorax, 22 without) were studied with high-resolution computed tomography (HRCT) of lungs. When the result was abnormal lung function testing was also performed. Bullous lung lesions were identified by CT in 13 individuals, 7 affected and 6 unaffected. One individual had bullae detected during lung operation. All those who had had one or more episodes of pneumothorax had these changes. The number of bullae varied from 1 to 30 and the diameter from 1 to 6 cm. While 93% (14/15) of known gene carriers had the lung lesions, lung function testing was normal in all but three. Including the data on occurrence of spontaneous pneumothorax and HRCT findings, autosomal dominant inheritance was seen in the pedigree. The clinical findings and the recognition of autosomal dominant inheritance enabled the performance of genome-wide linkage analyses in this family to identify a susceptibility locus for PSP. While the results are presently being analyzed we already can conclude that we have identified an inherited form of spontaneous pneumothorax caused by highly penetrant bullous lung lesions in a family of Finnish origin. The pedigree clearly shows autosomal dominant inheritance with high penetrance in gene carriers. Thus, individuals at risk of familial spontaneous pneumothorax can be identified with HRCT. This is of importance for making the correct diagnosis in case of unexpected chest pain.

Nutritional profile and folate plasma level in women in Argentina, Guatemala, and Venezuela, and prevalence of neural tube defects. *T. Mosby*¹, *L.H. Clemens*³, *D. Slawson*³, *M. Tolarova*². 1) Pediatric St. Jude Children's Research Hospital 332 N. Lauderdale, Memphis, TN; 2) Craniofacial Genetics, Department of Orthodontics, University of the Pacific School of Dentistry, San Francisco, CA; 3) University of Memphis, Memphis, TN.

It is well understood nowadays, that a vast majority of neural tube defects (NTD) are caused by a combination of genetic factors creating a susceptibility that is triggered by an environmental factors. Among environmental factors, deficiency in mothers nutrition, and specifically low daily intake of folate seems to be a major player. It has been clearly demonstrated that birth prevalence of NTD has significantly decreased in the USA and Canada after introduction of the mandatory fortification of grains with Folic Acid (FA). In March 1996, the FDA authorized addition of synthetic folic acid (FA) to enriched grain products at the level of 140 g per 100 grams of grain, with a mandatory compliance to be achieved by January 1998. The increase in average daily intake of FA in the US population was estimated to be 100-200 g of FA daily in the pre-mandatory period. However, the actual measured intake of FA from fortified foods ranged from 215 to 240 g/day. In the present study, a prediction formula by Wald et al. (2002) was used to calculate how different levels of FA fortification might decrease the birth prevalence of NTD in Argentina, Venezuela, and Guatemala. Detailed nutritional information and blood specimens were collected from subpopulations of 342 women of childbearing age in Argentina (n=110), Venezuela (n=85), and Guatemala (n=147), and daily folate intake and plasma folate levels were analyzed. Based on the results a decrease of birth prevalence of NTD in dependence on increase of FA intake was calculated. This study demonstrates that decrease of birth prevalence of NTD by approximately 50% could be achieved by increasing the daily FA intake by 0.4 mg/day in Argentina, by 0.8 mg/day in Venezuela, and by 1.0 mg/day in Guatemala. These results can be used for future recommendation of sufficient fortification levels in those countries. The fieldwork for this study was supported by Rotaplast International Inc.

Evolution of a complex phenotype in a unique patient with a paternal uniparental disomy for every chromosome cell line and a normal biparental inheritance cell line. *C. Bryke, A. Garber, J. Israel.* Comprehensive Genetic Services, Milwaukee, WI.

We previously described an infant with mosaic paternal isodisomy (UPID) for every chromosome and normal biparental inheritance (BPI) (ASHG 67(4):366 #2051 (2000)). She was a growth retarded baby with an ASD, hepatomegaly, macrodactyly, hypotonia and developmental delay. UPID for every chromosome, proven by STR analysis, was found in peripheral blood, adrenal, liver and uroepithelial cells. Buccal cells had normal BPI. The UPID and BPI lines had the same paternal haplotype. The phenotype of known patUPD syndromes has evolved in this child who is now 5. At birth she had transient neonatal diabetes (patUPD6). At 8 months she had features of McCune-Albright syndrome and underwent adrenalectomy for corticosteroid excess due to nodular adrenal hyperplasia (patUPD20). At 18 months a liver mass and elevated AFP level led to hepatic lobectomy for focal nodular hyperplasia. This finding and limb asymmetry suggested the diagnosis of Beckwith-Wiedemann syndrome (patUPD11). Her unsteady gait, lack of speech, blue eyes, blond hair, significant developmental delay, and staring spells are consistent with Angelman syndrome (patUPD15). Her prominent philtrum and the unusual radiographic appearance of her chest with caudal bowing of the anterior ribs and cranial bowing of the posterior ribs are likely the result of patUPD14. She also has developed features that do not fit well with the UPD syndromes described to date. Her large capillary hemangioma, striking labial enlargement, and hypertrophic lower extremity, ear, and toe could be due to Klippel-Trenaunay-Weber (KTW) syndrome. The extensive hyper- and hypopigmentation on her chest is compatible with Hypomelanosis of Ito (HI). These findings suggest that KTW and HI may be due to patUPD. Several co-existent UPDs, mosaicism, and possible paternal homozygous mutant recessive genes all contribute to this patient's complex phenotype. The lymphoblastoid UPID and BPI cell lines which have been isolated from this patient will be an invaluable resource for identifying maternally imprinted genes and differentially expressed genes.

Radiological and Clinical Comparisons in Fragile X-Associated Tremor Ataxia Syndrome. *S. Cohen*^{1, 2}, *J. Adams*², *D. Hess*², *S. Rivera*², *L. Gane*², *S. Jacquemont*², *F. Tassone*³, *P. Hagerman*³, *R. Hagerman*². 1) Human Development, UC Davis, Davis, CA; 2) M.I.N.D. Institute, UC Davis Medical Center, Sacramento, CA; 3) Biological Chemistry, UC Davis Medical Center, Davis, CA.

Introduction: Older adult males (>50 years) with the FMR1 premutation (55-200 CGG repeats) have been identified as being at high risk for developing a neurological disorder called fragile X-associated tremor/ataxia syndrome (FXTAS). Features of FXTAS include cerebellar ataxia, intention tremor, cognitive decline, and brain atrophy with associated white-matter disease (wmh). This study compares radiological changes as seen in structural MRI images, as well as clinical measures of performance and verbal ability, across 3 groups of older males premutation carriers with and without FXTAS, as well as with age-matched controls who do not carry the FMR1 mutation. **Subjects:** 31 symptomatic (FXTAS) and 8 asymptomatic male premutation carriers (ages, 51-91; M=68.82), and 19 male controls without the premutation allele (ages, 51-79; M=64.11). Subjects participated in a battery of neurological and neuropsychological tests, structural MRI, and provided blood for molecular analyses. **Results:** Compared with the asymptomatic (carrier) and control groups, the FXTAS group had significantly increased whole brain wmh [Kruskal-Wallis (KW)=8.317, p<0.01] and cerebellar wmh (KW=11.039, p<0.01), and decreased whole brain volume (KW = 18.598, p<0.001). FSIQ (F=4.383, p< 0.05), and PIQ (F=5.869, p<0.01) were significantly lower for those with FXTAS compared with typical controls, but not with unaffected premutation carriers. With controlling for age, VIQ was not significantly different across groups. Compared with typical controls, but not with unaffected carriers, those with FXTAS also had significantly worse motor planning and control as measured via the motor portion of the UPDRS (F=3.975, p<0.05), and the ICARS (F=3.877, p<0.05). **Discussion:** FXTAS males have significant volume loss and white matter hyperintensity in the brain. They also have significant cognitive and motor deficits compared to controls. Verbal language fluency and comprehension seem to be less compromised in FXTAS males than performance ability.

A combined approach to factor VIII genetic analysis in haemophilia A patients. *A.D. Laurie¹, J.R. Harraway¹, C.R. Sheen¹, M.P. Smith², P.M. George¹.* 1) Molecular Pathology, Canterbury Health, Christchurch, New Zealand; 2) Department of Haematology, Christchurch Hospital, New Zealand.

Haemophilia A is an inherited bleeding disorder caused by mutations in the factor VIII gene, located on the long arm of the X-chromosome. In our laboratory we are tackling the research and diagnostic testing for factor VIII gene mutations from several different angles. To improve the reliability of screening for the common intron 22 inversion, which accounts for ca. 20% of all cases of haemophilia A, we are developing a FISH-based approach which we hope will replace the current long-range PCR technique. We have also developed a microsatellite linkage method using five markers on the X-chromosome which can be used to track affected alleles when the factor VIII gene mutation is not known. Both of these techniques are suitable for single cell analysis and may be used in pre-implantation genetic diagnosis (PGD), a service which we hope to offer in the near future. In addition, we routinely screen haemophilia A patients for an inversion in intron 1, and perform a complete screen of all 26 exons using denaturing high performance liquid chromatography (DHPLC) which detects missense mutations and small insertions/deletions. Exons with suspicious DHPLC profiles are further analysed by nucleotide sequencing to identify the mutation. In a cohort of 21 local haemophilia A patients who were negative for the intron 22 inversion, we detected the intron 1 inversion in three patients and missense mutations in 12 patients. A female patient who has severe haemophilia A was heterozygous for a single adenine insertion in exon 14. Our work utilises a diverse range of molecular techniques to identify and track factor VIII gene mutations in our local patients, providing valuable support to clinicians and building a useful resource for research on haemophilia A.

COMPLETE ANDROGEN INSENSITIVITY SYNDROME DUE TO AR GENE DIFFERENT MUTATIONS IN AUNT AND NIECES: CASE REPORT. *E.Silveira. Ramos, P.L. Takeuchi, F.C. Dolabella, F.M. Araujo, L. Martelli, S.A. Santos, M.A.V. Calerani, C. Vretos.* Department of Genetics, School of Medicine of University of São Paulo, Ribeirão Preto, São Paulo, Brazil.

The androgen insensitivity syndrome (AIS) causes deficiency or absence of virilization in 46,XY individuals with normal androgen production and metabolism. AIS is a X-linked disorder, mapped at Xq11-12, estimated to be present in 1:20,000-64,000 male births. Variable phenotypic expression has allowed the classification into complete forms (CAIS) and partial forms (PAIS). A variety of different strategies for mutational screening of the androgen receptor gene (AR) have revealed over 300 mutations in AIS. The heterogeneity in the phenotypic expression of AIS is due to a variety of mutations in all eight exons in the AR gene. The authors reported one family (an aunt, three nieces, and the child of a niece who is a normal carrier) referred to the Multidisciplinary Staff of Studies of Sexual Determination and Differentiation at the University Hospital - School of Medicine of Ribeirão Preto at University of São Paulo, in which there were five cases of the CAIS. The clinical exam showed female external genitalia in all cases and after gonadectomy in four of them the histological studies showed the presence of bilateral testis. One patient, the youngest one, presented inguinal hernia at birth and underwent surgical procedure in another service. Cytogenetic analysis showed 46,XY karyotypes. Molecular studies by PCR-SSCP of AR gene (exons 2, 3, 4, 5, and 6) were made and alteration in exon 3 only for one patient (the aunt) was found. Molecular biology tests for exons 1, 7, and 8 for AR gene are in process. To verify the discordant mutation in the patient, the pedigree was verified and we noted that the original family was, in fact, composed by a marriage between two distinct families that have discordant mutations in AR gene. The world incidence of CAIS is estimated in 1:64,000; therefore the frequency expected to occur a marriage between two affected distinct families is almost statistically insignificant.

Allele distribution of FMR1 gene in Korean women. *H. Ryu*¹, *D.J. Kim*², *S.Y. Park*², *S.M. Kim*², *J.M. Kim*², *J.W. Kim*², *M.H. Lee*², *J.H. Kim*¹, *M.Y. Kim*¹. 1) Dept OB/GYN, Samsung Cheil Hos, Sungkyunkwan Univ, Seoul, Korea; 2) Department of Obstetrics and Gynecology, Sungkyunkwan University School of Medicine, Seoul, Korea.

Fragile X syndrome is one of the most common forms of inherited mental retardation and is caused by the expansion of CGG trinucleotide repeats in the FMR1 gene. Diagnostic test for Fragile X syndrome should ideally combine both PCR and Southern blot analysis. The objective of this study is to ascertain the distribution of FMR1 CGG repeat in general Korean women. We evaluated 700 unrelated Korean women who visited for antenatal care or preconceptional counseling. PCR analysis allowed an accurate determination of the repeat length in the normal and premutation range using fluorescence. When single PCR band was detected, we performed southern blot analysis for determination of homozygote and heterozygote with full mutation. In this study, twenty-five percent of tested women were homozygote. 95.3% of alleles had repeats ranging from 22 to 37 repeats. The most frequent allele (34.4 %) was the repeat of 29, followed by the 30 (24.8 %) and the 28 (6.8 %) repeat alleles. Eight cases were found to have repeat numbers in high borderline range (45~54 repeats). Our results provide a basic information for fragile X carrier screening in Korean population.

INSENSITIVITY ANDROGEN SYNDROME ASSOCIATED WITH MICROCEPHALY, MENTAL DEFICIENCY AND HYPOPLASY OF FOURTH METATARSI: CASE REPORT. *C. Vretos, P.L. Takeuchi, F.C. Dolabella, L.C. Caetano, S. Tucci Jr., E.S. Ramos.* Department of Genetics, University of Ribeirão Preto, São Paulo, Brazil.

Among all intersexuality pathologies, the androgen insensitivity syndrome (AIS) is an entity that causes deficiency or absence of virilization in 46,XY individuals with normal androgen production and metabolism. AIS is an X-linked disorder and estimated to be present in 1:20,000-64,000 male births. Variable phenotypic expression has allowed the classification into complete (CAIS) and partial forms (PAIS). While individuals with CAIS have female external genitalia, affected cases of PAIS have variable ambiguity. If reread as girls, both groups also undergo gonadectomy to eliminate the risk of gonadal malignance. The heterogeneity in the phenotypic expression of AIS is due to a variety of androgen gene mutations in all eight exons of the AR gene. The authors reported two sisters who parents were cousins with the partial form attempted at the Multidisciplinary Staff of Studies of the Sexual Determination and Differentiation of the University Hospital School of Medicine of Ribeirão Preto-University São Paulo. The clinical exam showed ambiguous external genitalia and after gonadectomy the histological studies presented bilateral testis in both cases. However, the youngest sister had another anomalies associated, as mental deficiency, microcephaly, dental abnormalities, cubitus valgus, fourth metatarsal hypoplasia, spina bifida in L5 and cervical scoliosis. Cytogenetic analysis showed 46,XY karyotypes. Molecular biology studies for AR gene (exons 2, 3, 4, 5 e 6) were made and no abnormal PCR-SSCP runner was found. Molecular biology tests of exons 1, 7, and 8 for AR gene are in process. The authors discuss that the alterations found in the youngest patient may have distinct etiology of androgen insensitivity syndrome.

Manitoba Oculotricooanal syndrome (MOTA) is not allelic to FRAS1 gene causing Fraser syndrome. E.

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Manitobaoculotricooanal syndrome (MOTA; OMIM 248450) is a rare autosomal recessive craniofacial malformation with hypertelorism and a variable combination of unilateral eye malformations, such as anophthalmia, obstruction of the nasolacrimal duct and upper eyelid coloboma, anterolateral hair and anal abnormalities (*Am. J. Med. Genet.* 1992;42(6).793-799). It was first reported in an inbred large Manitoba Indian family in central Canada and a recent follow-up of the family has revealed 6 further cases with a suspected seventh case from the Netherland. Here we present an autosomal recessive Turkish kindred with upper eyelid coloboma, agenesis/dysgenesis of eyebrows, wedge of scalp hair in fronto-temporal region extending to eyebrows, hypertelorism, depressed nose, ankyloblepharon filiforme totalis and anophthalmia. This family shows striking similarity to MOTA with overlapping features to autosomal recessive frontofacionasal dysostosis (FFND; OMIM 229400) and Fraser syndrome (OMIM 219000). Recently, mutations in FRAS1 gene which encodes a putative extracellular matrix protein has been reported as a cause of Fraser syndrome. Linkage analysis between the DNA markers, D4S2363, D4S2630, D4S2947, D4S3003 and the disease phenotype clearly excluded 4q21 region containing FRAS1 gene. Recent molecular studies in one Manitoba family with MOTA excluded mutations in the FRAS1 gene (personal communication, Anne Slavotinek, UCSF). Homozygosity mapping also excluded the following loci: FGFR1-3 on chromosomes 8p12, 10q26 and 4ptel, TWIST and IRF6 on chromosomes 7p21 and 1q32-q41 respectively.

Structural central nervous system (CNS) anomalies in Kabuki make-up syndrome. *T. Ben-Omran, A. Teebi.*
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Kabuki make-up syndrome (KMS) [OMIM# 147920] is a rare multiple congenital anomaly retardation (MCA/MR) syndrome commonly diagnosed at a genetic clinic. Duplication of chromosome 8p22-8p23.1 was recently demonstrated in multiple cases of KMS from different races, suggesting a possible common etiologic basis. KMS is characterized by a peculiar facial appearance with long palpebral fissures and long eyelashes, eversion of the lower lateral eyelid, arched and notched eyebrows. Other features include prominent and anteverted ears, as well as a characteristic dermatoglyphic patterns with prominent fingertip pads. Mental retardation has been found in over 90% of patients. Since the facial appearance often reflects the developing brain, it is not unexpected to observe a relatively higher frequency of CNS anomalies compared with disorders with minimal facial dysmorphism. CNS anomalies have been noted in approximately 30% of patients with KMS, the majority of which have microcephaly. Seizures have been seen in 50% of all patients. A spectrum of structural CNS anomalies has been reported including microcephaly with no apparent structural defects, enlarged ventricles, cysts, hydrocephalus, periventricular changes, polymicrogyria, heterotopia, calcification, and chiari I malformation. Here we report on two unrelated patients with typical Kabuki make-up syndrome and structural CNS anomalies. Case 1: 11-year-old girl with the typical clinical findings of KMS and Chiari I malformation. Case 2: 3 year-old boy with typical clinical features of KMS and Dandy Walker variant. Literature pertaining to the association of Kabuki make-up syndrome with structural CNS anomalies is reviewed, and the importance of brain imaging studies in the management of patients with KMS is emphasized.

De novo deletion 6q25.1q25.3 in a girl with normal development. *M.T. Carter¹, M.J. Huggins², J. Xu³, M.J.M. Nowaczyk^{3,4}.* 1) MD Program, McMaster University, Hamilton, Ontario, Canada; 2) Department of Pathology and Molecular Medicine, and Hamilton Regional Laboratory Medicine Program, Hamilton, Canada; 3) Department of Obstetrics and Gynecology, McMaster University, Hamilton, Canada; 4) Department of Pediatrics, McMaster University, Hamilton, Canada.

We report a girl with a de novo interstitial deletion of 6q25.1q25.3 and with minimal developmental delays at age 32 months. Her mother was referred to prenatal genetics service at 32 weeks gestation when a sonographic evaluation showed intrauterine growth retardation, small chest circumference and shortened long bones, a left axial displacement of the heart chambers, and a large patent foramen ovale/secundum atrial septal defect. A genetic amniocentesis was performed and showed an abnormality of 6q. Minor anomalies observed after delivery observed in this girl include triangular face, frontal bossing with metopic prominence, short and upward-slanting palpebral fissures, asymmetry of upper eyelids, shallow orbits, prominent inferior orbital crease, wide mouth, long and flat philtrum, redundant skin and wrinkled skin, joint laxity, small thorax, proximal limb shortening and a deep and wide sacral dimple. She has pigmentary anomalies of retinas and hypoplasia of external genitalia. To rule out possibility of this abnormal chromosome 6 resulted from a translocation, FISH of metaphase chromosomes using whole chromosome paint (wcp) 6 was conducted. The wcp 6 painted uniformly along the entire length of chromosome 6 including the distal 6q and did not hybridize to any other chromosomes, indicative of no translocation involved. The parents have a normal karyotype and thus the proposita has a de novo abnormal karyotype 46,XX, del(6)(q25.1q25.3). In patients with 6q deletion reported to date mental retardation has been a consistent finding; cardiac defects, eye abnormalities and cleft palate are found commonly. To our knowledge this is the first patient with interstitial deletion of 6q25 who presents minimal delays in psychomotor development. Disruption of an estrogen receptor gene *ESR1*, which maps to 6q25.1, may be responsible for the genital and retinal anomalies observed in patients with the 6q deletions.

New syndrome with features of cleidocranial dysplasia with de novo three-way chromosome translocation, 46,XY,t(4;6;21)(p16;p21.1;q21). *K. Casas, P.L. Wilson, J.L. Lee, R. Muneer, S. Li, J.J. Mulvihill.* Department of Pediatrics, Section of Genetics, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104.

In the past, chromosome translocations in persons with a mendelian trait have been clues to gene mapping. In 1992, our predecessors discovered a 3-way translocation in a 3-year old boy with an uncharacterized syndrome with multiple dysmorphism. The family sought re-evaluation as he was turning 11 years old and had bone pain. Repeat cytogenetic analysis with FISH identified apparently balanced translocations: chromosome 4 (4p16->4pter) translocated onto chromosome 6 at sub-band 6p21.1; chromosome 6 (6p21.1->6pter) translocated onto chromosome 21 at the middle of band 21q21; and chromosome 21 (21q21->21qter) onto chromosome 4 at band 4p16. Both parents had normal karyotypes. Family history was negative for fetal wastage, consanguinity, and similarly affected individuals. Physical examination revealed a male with hypermobile joints, kyphoscoliosis, pes planus, hyperplastic 4th and 5th toes, soft skin, two cafe-au-lait macules, umbilical hernia, and easy bruisability. Skeletal radiographs showed delayed fontanel closure, hypoplastic clavicles, 11 ribs, hypoplastic pubic rami, and widened symphysis pubis. The gene maps adjacent to all breakpoints were screened for genes implicated in skeletal dysplasia, connective tissue disorders, and syndromes. In comparing the patient's features to traits of the above conditions, the best association was with cleidocranial dysplasia. Involvement of RUNX2 in the breakpoint at 6p21.1 is likely. Connective tissue features in the patient that are not typical of cleidocranial dysplasia suggest involvement of a second gene in his unique syndrome. The patient and family were counseled regarding reproductive risks and the possible adverse clinical effects of his chromosomal translocations.

Diagnosis of microdeletion syndromes by Real-Time PCR based on the measurement of gene copy number. *Y.H. Cho, E.S. Shin, J.H. Kyhm, C.H. Lee.* Dept Medical Genetics, College of Medicine, Hanyang University, Seoul 133-791, Korea.

Deletion or duplication of genes or genomic regions have been identified as a genetic cause of various disorders, including mental retardation. To facilitate the detection of such cases, different techniques such as FISH, polymorphic marker, CGH and real-time PCR have been developed. Gene copy number analysis by real-time PCR can be targeted directly to a selected subregion of the gene of interest, and does not depend on the availability of informative flanking markers or parental DNA. There are some recurring chromosomal microdeletion syndromes causing MR such as Prader-Willi, Angelman, DiGeorge and Williams which were 5~10% of chromosomal microdeletion syndromes and idiopathic MR and would not have been detected by conventional cytogenetic analysis in addition. In order to determine the gene copy number of the 4 target genes in MR group by real-time quantitative PCR, conventional cytogenetic analysis and fragile X detection were performed previously in 102 MR group and 47 normal control group (GTG-banding(-) and Fragile X(-)). Real-time quantitative PCR using SYBR Green I has been used in this study. The relative gene copy number was quantified by the comparative Ct method. Five cases of DiGeorge syndrome patients were confirmed by FISH method and its range of target gene ratio was 0.36-0.60. The target gene ratio in normal controls ranged from 0.74-1.37, 0.74-1.39, 0.73-1.35 and 0.73-1.32 in DiGeorge, Williams, Prader-Willi and Angelman syndrome in order. The target gene ratio in MR group ranged from 0.73-1.35, 0.73-1.37, 0.73-1.27 and 0.73-1.35 in DiGeorge, Williams, Prader-Willi and Angelman syndrome in order. Eventhough, we could not detect any rearrangement of target regions in this MR group, this method is simple and reliable DNA diagnostic tool for the some recurring chromosomal microdeletion syndrome and permits of high sample throughput and can therefore be used in large-scale screening projects.

Evaluation of genotoxic effects of Tetradifon (an organochlorine pesticide: CAS N 116-29-0) in female wistar rats. *R. Badraoui, N.B. Abdelmoula, T. Rebai.* Laboratoire d'Histologie, faculte de medecine de sfax, Tunisia.

Tetradifon is a potent organochlorine acaricide with an estrogen like structure. It is used as an herbicide against a wide variety of vegetables. The wide spread use of this chemical is likely to pollute the environment. Only few studies have been reported for the evaluation of its short and long-term toxic effects including genotoxicity and carcinogenicity and there have been conflicting results in in vitro and in vivo test systems. In this work, we have evaluated tetradifon for its ability to induce genotoxic damages in female wistar rats. A single dose of 2430 mg/kg BW was administrated orally for 12 female rats. Twelve others rats, non traeted, served as a control. Animals were sacrificed at 6 and 12 weeks. Genetic toxicity studies were conduced in rats bone morrows, by chromosomal tests (sister-chromatid exchange (SCEs) and cytogenetics assays). The treated animals cells show a non statistical significant increase in either the frequency of SCEs and chromosomal aberrations (CAs) in comparison to the control rats. The oxidative stress status of treated and no treated animals have been also evaluated by assessment of lipid peroxidation (measurement of the thiobarbituric acid reacted substances (TBARS) as malondialdehyde (MDA)). There were a statistically significant difference between the 2 groups of animals suggesting that Tetradifon induce an oxidative stress. These results indicate that tetradifon is likely not directly genotoxic in female wistar rats. But we suggest that its inducting of an oxidative stress via oxydants defences may leads to indirect mutagenecity that should be evaluated by a series of in vivo genotoxicity assays (cytogenetics, micronucleus, comet assay).

Gene Expression Profiling at the Subnuclear Level in the Rat Hypothalamus and Hippocampus with High-throughput Multiplex RT-PCR. *G. Yue¹, M. Azaro¹, Q. Yang¹, G. Hu¹, M. Luo¹, R. Gao¹, R. Nagele³, J. Neubauer², J.Y. Li¹, K. Yin⁴, H. Li¹.* 1) Department of Molecular Genetics & Microbiology, CINJ/UMDNJ-RWJMS, New Brunswick, NJ 08903; 2) Department of Medicine, UMDNJ-RWJMS, New Brunswick, NJ 08903; 3) Department of Molecular Biology, UMDNJ-SOM, Stratford, NJ 08084; 4) Department of Cell Biology, UMDNJ-SOM, Stratford, NJ 08084.

Gene expression profiling at the subnuclear level of the hypothalamus is essential for understanding its subnuclear molecular interactions and functions. Laser capture microdissection was used to dissect rat brain cryosections. Sections between Bregma Scale -3.3 mm to -3.54 mm were used. Neurons from the hypothalamic arcuate nuclei, periventricular nuclei and dorsomedial nuclei were selected for the analysis. One hundred to 200 neurons were used from a nuclear region in a section for each RT-PCR. Neurons of the dentate gyrus of the hippocampus were also collected for comparison. More than 150 neural transmitters and function-related factors such as enzymes, receptors, and pathway-linking factors were selected for analysis. All amplicons are unique and surrounding at least one intron. Primers were designed with computer programs. One-step RT-PCR method was used to simultaneously amplify 164 distinct mRNA species (including controls) by multiplex RT-PCR. RT-PCR products were used as template to generate single-stranded DNA (ssDNA) with specific nested primers. The ssDNA was analyzed with the microarray and single-base extension methods. Out of the 164 mRNA species, 70 (42%) were detected from arcuate nuclei, 65 (40%) from periventricular nuclei, 73 (44.5%) from dorsomedial nuclei, and 71 (43%) from dentate gyrus. The signature gene expression in the dentate gyrus of the hippocampus was consistent with previous studies.

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CyDAS: a Cytogenetic Data Analysis System. *B. Hiller, J. Bradtke, H. Balz, H. Rieder.* Institut fuer Klinische Genetik, Philipps-Universitaet Marburg, Marburg, Germany.

Cytogenetic analysis is a valuable tool in genetic tumor diagnosis and research. Its results are communicated using ISCN formulae. But evaluation of large data sets is hampered by a lack of computer programs which can deal with ISCN data. CyDAS - which is available both online (www.cydias.org) and as a desk top version - can analyse ISCN data and extracts virtually all information contained in an ISCN formula. Furthermore, it calculates meta-information like break points, structural aberrations, gains and losses, dependencies between aberrations and karyotype evolution pathways. ISCN formulae are checked for syntax and content. This is a very important feature, since in data from the Mitelman database, some 10% of entries from 1995 or later were found to contain errors. Typical errors are: non-existing break points, wrong description of derivative chromosomes, insufficient information like missing break points or missing aberrations in derivative chromosomes, or unacceptable break points for translocations or dicentric chromosomes. CyDAS was used to analyse all 883 entries of patients with adenoma of the kidney from the Mitelman database for recurring chromosome changes. In 827 entries without karyotype errors breaks were most frequent at 3p11~21, 5q22, 1q21, Xp11 and 3q21. Gains were most frequently recorded at chromosomes 7 and 5q, and losses were most frequent at chromosomes 3p and Y. Dependency analysis of aberrations at the ISCN level showed that karyotypes containing at least one of +12,+16,+17, or +20 were most likely to contain also +7 (probability >0.7), followed by -Y (probability >0.45). Karyotype evolution analysis revealed +7 as the major start point, followed quite randomly by +17, +16, +12, +20 and -Y; other important pathways start with -Y followed by either +7 or -14, and with -14 followed by either -9, +7, or -3. All data were visualized by graphics. Overall, CyDAS proved to be a very useful tool to check karyotypes for errors and to ensure high quality of cytogenetic data for further analyses. Hot spots of genomic rearrangements and pathways of karyotype evolution may easily be identified. Supported by the DFG (Ri1123/2-1).

Identification of discrete chromosomal deletion by binary recursive partitioning of microarray differential expression data. *X. Zhou*¹, *S.W. Cole*², *N.P. Rao*³, *Z. Chen*⁴, *D.T. Wong*^{1,5,6}. 1) Dental Research Inst, School of Dentistry; 2) Division of Hematology-Oncology, Department of Medicine; 3) Department of Pathology & Laboratory Medicine; 4) Department of Human Genetics, David Geffen School of Medicine; 5) Jonsson Comprehensive Cancer Center; 6) Molecular Biology Institute, University of California at Los Angeles, Los Angeles, CA.

Gene copy-number abnormalities (CNA) are characteristic of solid tumors, and also found in association with congenital multiple anomalies and mental retardation. The ultimate impact of copy number abnormalities is manifested by the altered expression of the encoded genes. We have previously developed statistical methods for the detection of chromosomal aberrations using microarray expression data (Zhou et al., *Hum. Genet.*, 2004 114(5):464-467). In this study, we adapted these analytic techniques to detect small chromosomal deletions based on differential gene expression data. Using three cell lines with known chromosomal deletions as model system, we compared mRNA expression in these cells with that observed in diploid cell lines of matched tissue origin. The results showed that genes from deleted chromosomal regions are substantially over-represented ($p < .000001$ by chi-square) by genes identified as under-expressed in deletion cell lines, relative to normal matching cells. Using a likelihood-based statistical model, we localized the breakpoints of the chromosomal deletion in each cell line. In one of such cell lines, our analyses refined the previously identified chromosomal deletion region. We identified a 10p14-10p12 deletion instead of the previously identified 10pter-10p12 deletion, which was further confirmed by fluorescence in situ hybridization (FISH). These data show that microarray differential expression data can be used to detect and map the boundaries of discrete and cryptic chromosomal loss and gains.

Mohr-Majewski syndrome :a new argument for a continuous spectrum between oro-facio-digital syndrome and short-rib syndrome. *F. Mochel¹, V. Mucignat², H. Ducou-Le Pointe³, F. Gold², M. Le Merrer¹.* 1) Genetic Department, Hopital Necker-Enfants Malades, Paris, France; 2) Neonatal Department, Hopital Armand Trousseau, Paris, France; 3) Radiology Department, Hopital Armand Trousseau, Paris, France.

We report on a male newborn that presents the complete spectrum of the clinical manifestations observed in both Mohr syndrome (hypertelorism, micrognathia, cleft lip and palate, lobulated tongue and hallucal polysyndactyly) and Majewski syndrome (short ribs, short mesomelic limbs, laryngeal stenosis and polysyndactyly). This new case supports the well-known hypothesis elaborated in 1978 by McKusick about a continuous spectrum between oro-facio-digital syndromes (OFDS) and short-rib (-polydactyly) syndromes (SRPS). However, the premature death of the patient at 9 months of age emphasizes the severity of the prognosis related to respiratory insufficiency, contrasting with normal psychomotor evolution in this case. Despite its highly suspected autosomal recessive inheritance, the basic molecular defect of OFDS type IV remains unknown.

Characterization of a new gene disrupted by a balanced translocation t(2;7)(q35;p22) in a fetus with polymicrogyria and nodular heterotopia. *V. Cantagrel¹, C. Missirian², C. Cardoso¹, N. Philip², D. Figarella-Branger³, A. Moncla², L. Villard¹.* 1) Inserm U491, Faculté de Médecine La Timone, Marseille, France; 2) Département de Génétique Médicale, Hôpital d'Enfants de La Timone, Marseille, France; 3) Biopathologie Nerveuse et Musculaire, Faculté de Médecine La Timone, Marseille, France.

Polymicrogyria (PMG) is a development defect of the human cerebral cortex which results in an excessive number of small gyri and an abnormal organisation of cortical cell layers. PMG often is accompanied by mental retardation and epilepsy. The etiology of this cortical dysplasia is heterogenous but it was recently shown that a genetic origin is definitely present in several cases. We present clinical, cytogenetic and molecular data for a 33 week-old female fetus who was terminated based on the presence of hydrocephaly in combination with a de novo balanced translocation. Detailed examination of the fetus did not reveal signs of infection or inflammation but shows partial syndactyly of digits and toes. Neuropathology showed periventricular astrocytic gliosis and hypervascularisation in addition to nodular heterotopia throughout the white matter. Examination of the cerebral cortex revealed polymicrogyria with a predominance in the posterior areas. The presence of PMG and nodular heterotopia suggests a neuronal migration or neuronal organisation defect. The simultaneous occurrence of a balanced rearrangement provides a unique opportunity to identify a potential gene defect responsible for this phenotype. In order to precisely characterize the translocation, we have cloned and sequenced the two breakpoints. It is molecularly balanced with no loss or gain of genetic material. The 7p22 breakpoint region does not contain any gene. On the contrary, the 2q35 breakpoint disrupts the coding region of a novel gene. Its product has no significant homology to any other known protein. Our results make this new gene a good candidate to be responsible for the neuronal migration defect observed in the studied patient and the detailed analysis of its expression during development is currently underway.

Disruption of a new X-linked gene highly expressed in brain in a family with two mentally retarded males. *A.M. Lossi¹, V. Cantagrel¹, S. Boulanger², D. Depetris¹, M.G. Matti¹, J. Gecz³, C.E. Schwartz⁴, L. Van Maldergem², L. Villard¹.* 1) Inserm U491, Facult de Mdecine La Timone, Marseille, France; 2) Institut de Pathologie et de Gntique, Loverval, Belgium; 3) Department of Genetic Medicine, Women and Childrens Hospital, Adelaide, Australia; 4) Greenwood Genetic Center, One Gregor Mendel Circle, Greenwood, SC, USA.

Mental retardation (MR) affects 2 to 3% of the human population and up to 50% of these cases is genetically determined. Although several genes responsible for MR have been identified, a large number of cases is still not explained. We have identified a pericentric inversion of the X chromosome $inv(X)(p22.3;q13.2)$ segregating in a family where two male carriers have severe MR whereas female carriers are not affected. The molecular characterization of this inversion led us to identify two new genes which are disrupted by the breakpoints : KIAA2022 in Xq13.2 and P2RY8 in Xp22.3. KIAA2022 encodes a protein which lacks significant homology to any other known protein. It is highly expressed in the brain. P2RY8 is a member of the purine nucleotide G-protein coupled receptor gene family. It is located in the pseudo-autosomal region of the X chromosome. It is not expressed in brain. Because the haplo-insufficiency of P2RY8 in carrier mothers does not have a phenotypic effect, we propose that the severe MR of the affected males in this family is due to the absence of the KIAA2022 gene product. However, screening 20 probands from X-linked MR families did not reveal any mutations in KIAA2022. Given the large genetic heterogeneity of XLMR this result may not be unexpected. Nonetheless, the high expression of this gene in foetal brain and in the adult cerebral cortex is consistent with its speculated a role in brain development and/or cognitive function.

Deletion of 5q14-q21 associated with temporal periventricular heterotopia. *E. Pallesi¹, C. Missirian², A. Ramazzotti³, C. Raybaud⁴, B. Chabrol⁴, R. Guerrini³, A. Moncla², L. Villard¹, C. Cardoso¹.* 1) INSERM U491, Faculte de Medecine la Timone, Marseille, France; 2) Departement de Genetique Medicale, Hopital des enfants de la Timone, Marseille, France; 3) Institute of Child Neurology and Psychiatry, IRCCS Fondazione Stella Maris, Calambrone, Italy; 4) Departement de Neurologie, Hopital des enfants de la Timone, Marseille, France.

Periventricular Heterotopia (PH) is a heterogeneous disorder which is characterized by collection of neurons in the subependymal white matter. These neurons have failed to migrate in the developing cortex which would therefore be expected to have a defective organization. Two genes have been identified to cause PH. The FLNA gene, in Xq28, causes X-linked dominant classical bilateral periventricular nodular heterotopia (BPNH) and ARFGEF2, in 20q13, causes autosomal recessive BPNH associated with microcephaly. In our current project to identify new candidate genes involved in PH, we have studied a male patient with a de novo interstitial deletion 5q14-q21 resulting from an unbalanced translocation between chromosomes 1 and 5. His brain MRI shows heterotopic nodules within the temporal lobes which define a subtype of PH previously described by Li et al. 1997. In addition, our patient presents severe mental retardation, hypotonia, iris coloboma, facial dysmorphism and symptomatic epilepsy. CGH array and FISH analysis were used to precisely define and delineate the rearrangement. We have excluded the presence of a causative gene within the 1q31 breakpoint and we found 18 well characterized genes in the 15 Mb deleted region. RT-PCR expression analysis in human tissues and brain regions identified two candidate genes expressed either in the temporal lobes or throughout the brain. Sequencing analysis of these genes in sporadic PH patients with no mutations within FLNA and ARFGEF2 genes should allow the identification of the temporal PH causative gene, and provide new insights into the genetic and developmental basis of this cortical malformation.

Breakpoint mapping in a patient with an X;9 translocation, mental retardation and overgrowth. S.J.T.

Vermeulen¹, B. Menten¹, J. Depuydt¹, G. Mortier¹, A. De Paepe¹, J. Vandesomepele¹, N. Carter², F. Speleman¹. 1) Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium; 2) The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus Hinxton, Cambridge, United Kingdom.

Constitutional de novo chromosomal rearrangements in patients with mild to severe mental retardation and congenital abnormalities have been instrumental in the identification of disease genes. We present a patient with mental retardation, overgrowth and a de novo apparently balanced chromosomal rearrangement 46,XX,t(X;9)(q12;p13.3). Using FISH we were able to locate the Xq12 breakpoint within a BAC-clone within the gene encoding oligophrenin (OPHN1). This observation is reminiscent to the t(X;12) case reported by Bienvenu et al. (1997) which also involves loss of function of the OPHN1 gene. Moreover, mutations in the OPHN1 gene have subsequently been reported in patients with mental retardation, (Billuart et al., 1998). These observations strongly suggest that the Xq12 breakpoint is responsible for the mental retardation in our patient. Overgrowth has not been reported in patients with a mutation in or structural rearrangement of the OPHN1 gene. The 9p13.3 breakpoint was shown to be located within a gene dense region. The derivative chromosomes were flowsorted and the breakpoints cloned and sequenced. No evidence was observed for disruption of a coding sequence. Several genes including the carbonic anhydrase IX gene (CA9) are located within the immediate vicinity of the 9p13.3 breakpoint. Only the latter gene showed a significant overexpression in our patient. As CA9 overexpression is implicated in cell proliferation (Saarnio et al., 1998) we consider this gene as a candidate for the observed overgrowth in this patient. References: Bienvenu, T. et al. *Eur J Hum Genet* 5, 105-9 (1997), Billuart, P. et al. *Nature* 392, 923-6 (1998), Saarnio, J. et al. *J Histochem Cytochem* 46, 497-504 (1998).

Chromosomal mosaicism in the developing human brain: high aneuploidy incidence could explain neuronal loss during ontogenesis. *Y.B. Yurov¹, V.M. Vostrikov¹, V.V. Monakchov¹, I.Y. Iourov¹, S.G. Vorsanova².* 1) Dept Cytogenetics, NCMH, RAMS, Moscow, Russian Federation; 2) Institute of Pediatrics and Children Surgery Russian Ministry of Health, Moscow, Russia.

There are a limited number of molecular cytogenetic studies of the chromosome complement in the human brain. In the present study fluorescence in situ hybridization (FISH) was used for analysis of interphase chromosomes in human embryonic central nervous system (CNS) cells cultured in vitro. Ten organotypic cultures of embryonic human brain cells were analyzed. Ten post-mortem adult brain samples were used as control for aneuploidy scoring. From 1000 to 3000 hybridized cells were analyzed for each sample using three-color FISH. The level of aneuploidy (per individual chromosome) was in the range of 0.2-13%; in embryonic CNS cells (mean values for chromosome 1 - 5.0%; chromosome 9 - 6.2%; chromosomes 13 and 21 - 3.7%; chromosomes 14 and 22 - 2.8%; chromosome 15 - 3.0%; chromosome 16 - 4.9%; chromosomes 18 - 1.8%; chromosome X - 5.8%; chromosome Y - 5.7%). The level of aneuploidy in post-mortem brain cells was in the range of 0.1 - 1.2%. Our results indicate that embryonic CNS cells and adult brain cells are significantly different in the mosaic aneuploidy incidence. Therefore, we could propose that selective loss of neuronal cells with abnormal chromosome complement is taking place during early ontogenesis. However, relatively small, but significant, amount of aneuploid neurons could survive and exist in the adult brain. High level of chromosomal aneuploidy in embryonic human neuronal cells could, therefore, leads to genetic mosaicism in fetal and, probably, adult CNS. Cryptic chromosomal mosaicism of the brain have no a distinctive phenotypic appearance; however it could affect the development as well as functions of the brain. We are also suggesting that the abnormal chromosome complement in neurons of human brain may have a relevance to many neuropsychiatric diseases. Supported in parts by grant Copernicus 2 and INTAS-03-4060.

Correlation between immunohistochemistry (Hercep Test) and FISH for HER-2 in breast cancer patients In Taiwan. *C. Liu*¹, *B. Wang*², *W. Tsai*², *K. Yeh*³, *H. Chang*³, *C. Yeh*³, *S. Chang*². 1) Vascular and Genomic Research Center, Changhua Christian Hospital, Changhua, Taiwan; 2) Ctr Medical Genetics, Changhua Christian Hospital, Taiwan; 3) Dept Pathology, Changhua Christian Hospital, Taiwan, Taiwan.

Accurate diagnostic assessment of HER-2 is essential for the appropriate application of the humanized anti-HER-2 monoclonal antibody trastuzumab (Herceptin) to the treatment of patients with metastatic breast cancer. The diagnostic test needs to be applicable to archival, fixed tissue removed at excision. We compared the assessment of HER-2 by immunohistochemistry (IHC; Hercep Test) and FISH (fluorescence in situ hybridization) in 60 breast carcinomas from patients being considered for trastuzumab therapy. 0/20 IHC 0/1+ were FISH positive, 1 of 20 (5%) IHC 3+ tumors were FISH negative. Overall, 48% of these IHC /2+ were FISH positive. These data support an algorithm in which FISH testing is restricted to IHC 2+ tumors in reference centers. We conclude that this FISH approach is practical in screening patients for Herceptin treatment, but the results may not extrapolate to laboratories with less experience or using different methodologies.

Subtelomeric deletions of chromosome 6p; molecular and cytogenetic characterization of 6 new cases in 3 families with phenotypic overlap with Ritscher-Schinzel (3C) Syndrome. C. DeScipio¹, L. Schneider¹, T.L. Young², N. Wasserman¹, D. Yaeger¹, P.G. Wheeler⁴, M.S. Williams⁵, R. Geschwind¹, A.E. Chudley⁶, J. Saraiva⁷, A.A.G.L. Schinzel⁸, A. Toutain⁹, N.B. Spinner^{1,3}, I.D. Krantz¹. 1) Division of Human Genetics; 2) Division of Ophthalmology; 3) Clinical Labs, The Children's Hospital of Philadelphia, Philadelphia, PA; 4) New England Medical Center, Tufts University School of Medicine, Boston, MA; 5) Gundersen Lutheran Medical Center, La Crosse, WI; 6) Section of Genetics and Metabolism, Children's Hospital and University of Manitoba, Winnipeg, Manitoba; 7) Servio de Génética, Hospital Pediàtrico de Coimbra, Portugal; 8) Institute of Medical Genetics, University of Zürich, Zürich, Switzerland; 9) Centre Hospitalier Universitaire de Tours, France.

We describe 6 cases of subtelomeric chromosome 6p microdeletions from 3 unrelated families; each has clinical findings consistent with the cranio-cerebello-cardiac (3C or Ritscher-Schinzel) syndrome consisting of facial dysmorphism, congenital heart defects (primarily septal defects), Dandy-Walker malformations, and hearing loss. Due to the phenotypic overlap of 3C syndrome with these 6 cases and other 6p25 deletions reported in the literature, we propose that microdeletions of 6p25 or mutations of a gene within this region may be responsible for 3C syndrome. We have molecularly and cytogenetically defined a critical region containing 7 genes spanning 1.3 Mb, delineating the smallest deletion of this region reported to date. In order to determine whether 6p is commonly deleted in 3C syndrome, we screened seven unrelated 3C individuals for deletions in this interval and none were detected. Direct sequencing of two forkhead genes (*FOXC1* and *FOXF2*), from within this critical region was carried out in the seven 3C probands. No disease-causing mutations were identified. Mutational analysis of the remaining 5 genes in the critical region is underway. While the phenotypic overlap between the 6p subtelomeric deletion syndrome and 3C syndrome is fairly striking it is possible that they may be phenocopies of each other.

Molecular characterisation of a translocation associated with hypoplastic left heart. *H. Gill¹, A. Davies¹, G. Sharland¹, B. Ng², N. Carter², M. Splitt¹, J. Simpson¹, C. Mackie Ogilvie¹, R. Roberts¹.* 1) Division of Medical and Molecular Genetics, Guy's Hospital, London United Kingdom; 2) The Wellcome Trust Sanger Institute, Cambridge.

AIMS We identified a proband with HLH and *de novo*, apparently balanced, reciprocal translocation. We aimed to fine map the translocation breakpoints, characterise them at the molecular level, and investigate any possible candidate genes in these intervals.

METHODS Fluorescent in situ hybridization was undertaken to narrow the breakpoints, utilising first bacterial artificial chromosomes and then fosmids as probes. Further narrowing of the region involved polymerase chain reaction (PCR) analysis of derivative chromosomes purified using a fluorescence-activated cell sorter. Finally, direct sequencing was used to confirm the exact breakpoint.

RESULTS Our work has revealed a complex chromosomal rearrangement with four distinct breakpoints on two chromosomes. Three of these breaks occurred within a 4-Mb region of chromosome 1; the fourth is on chromosome 3. We have characterised all four breakpoints at the molecular level.

DISCUSSION The rearrangement is likely to have arisen by a combined inversion of material on chromosome 1 and translocation between 1 and 3, either as a concerted event or as two sequential ones. Neither rearrangement was observed in the proband's parents. Although none of the breakpoints disrupt a known gene, two of them lie within a few kilobases of three candidate disease genes. One is known to be disrupted in an autosomal recessive encephalopathy. The others encode a serotonin receptor and an orphan steroid hormone receptor, both expressed in fetal heart. As the effects of dominant mutations in the encephalopathy gene are unknown, we consider all three genes as potential players in the aetiology of HLH in this individual.

Ongoing work: We are currently undertaking studies to investigate any alteration in function and/or expression levels in the proband in order to explain the disease mechanism.

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Molecular analysis in Williams syndrome in a sample of Mexican patients. *S. Cuevas, C. Venegas, S. Kofman.*
Genetica, Hospital General de Mexico, Mexico D.F., Mexico.

Williams syndrome (WS) is a contiguous gene deletion disorder due to deletion of 7q11.23 region. It occurs in 1 of 20,000-50,000 new born. Clinically, the classical WS phenotype is characterized by elastin arteriopathy, supraaortic stenosis, connective tissue anomalies, particular facial appearance, growth and psychomotor retardation with impaired visuo spatial cognition and specific language as well as behavior anomalies. Around of 95% patients with WS present deletion of the 7q11.23 region. The aim of the present study was to identify through FISH analysis the critical region of WS (WSCR) in a sample of Mexican patients. Most patients with classical phenotype harbored the WSCR deletion while patients with atypical phenotype had no deletion. Only one case with apparently classical phenotype showed no deletion of the WSCR. The occurrence of WSCR deletion was similar to that reported in the literature. We state the importance of performance molecular studies in the patients with probable WS to offer and correct genetic counseling.

Towards a first genotype-phenotype correlation of small supernumerary marker chromosomes (sSMC). *T. Liehr, K. Mrasek, U. Claussen, H. Starke.* Institute of Human Genetics and Anthropology, Jena, Germany.

Small supernumerary marker chromosomes (sSMC) are present in 0.043% of newborn. In about 30% of sSMC carriers an abnormal phenotype is observed. Clinical outcome of sSMC presence is difficult to predict as different phenotypic consequences can appear due to (i) differences in euchromatic DNA-content, (ii) uniparental disomy (UPD) of the sSMCs homologous chromosomes, and/or (iii) different degrees of mosaicism. We did own studies on >150 cases with sSMC and performed a review of the literature (i.e. presently 1505 cases with sSMC) available at http://mti-n.mti.uni-jena.de/~huwww/MOL_ZYTO/sSMC.htm. A first genotype/phenotype correlation for sSMC was deduced from that. Thus, small proximal trisomies of Xp, Xq, 1p, 1q, 2p, 4q, 5p, 6q, 7q, 8p, 8q, 12p, 12q, 14q, 17q, 18q, 19q, 20p, 20q, lead to clinical manifestations, while small partial proximal trisomies of 2q, 5q, 7p, 15q, 17p, 18p, 21q, 22q may not be associated with significant clinical symptoms. No general correlation could be found in connection with mosaicism of sSMC (47,+mar/46) and clinical symptoms. Recent own studies using new centromere and subcentromer-specific probe-sets ([sub]cenM-FISH; Starke et al., 2003, Hum Genet 114:51-67) gave evidence that this may be at least in parts due to the fact, that many patients do have different derivative-types of a sSMC however, these could not be detected and characterized before the development of subcenM-FISH. In summary, almost 50 year after description of the first sSMC, molecular cytogenetics provides finally approaches for their comprehensive characterization. The new knowledge coming from that will lead to an improved genetic counseling of cases with de novo sSMC. Supported by the Dr. Robert Pflieger-Stiftung.

Sex-preferential hotspots for non-allelic homologous recombination within segmental duplications in Williams-Beuren syndrome deletions. *N. Rivera, J. Lucena, M. Bayes, L.A. Perez Jurado.* Genetics Unit, Dept. of Experimental Sciences Universitat Pompeu Fabra, Barcelona, Spain.

The sporadic occurrence of Williams-Beuren syndrome (WBS) in most cases indicates a high rate of de novo deletion formation at 7q11.23. Most WBS deletions arise from non-allelic homologous recombination (NAHR) between two specific blocks (B) of region-specific segmental duplications (SDs) that show 99.5% sequence identity over 105 kb. Both, intrachromosomal (1/3) as well as interchromosomal (2/3) recombination events have been reported. Heterozygosity for large chromosomal polymorphisms such as paracentric inversions between SDs, appears to further predispose to chromosome misalignment in some progenitors of WBS patients. To define the region of exchange in WBS deletions, we have analyzed by dosage multiple paralogous sequence variants, until being able to amplify by PCR and sequence the novel junction fragments from the recombinant WBS-BlockB. The previously defined NAHR hotspot (positional preference for strand exchange) within block B, has been narrowed-down to a 1,4 kb region of complete sequence identity. Deletions with breakpoints at this site occur in 1/3 of cases with no parental predisposing allele and show clear biases in favor of paternal and intrachromosomal origin. Two additional NAHR hotspots located within the final 38 kb region of block B, account for all WBS deletions (n=18) in which the transmitting progenitor is an inversion-carrier. Seventy-five percent (6/8) of breakpoints of paternal origin mapped in a 950 bp hotspot, while 90% (9/10) of maternal crossovers were located in a different 1,4 kb hotspot, thus supporting the hypothesis that two distinct sex-dependent mechanisms are involved (P=0.02). Alu repeats and other putative recombination stimulating sequence are located inside and around the hotspots. Therefore, as shown for other genomic disorders, NAHR between SDs does not occur randomly; instead, several parent-of-origin-preferential recombination hotspots can be identified within the SDs.

FISH-mapping of telomeric 14q32 deletions: is there a seizure gene? *K. Schlade-Bartusiak¹, T. Costa², A.M. Summers³, D.W. Cox¹*. 1) Medical Genetics, University of Alberta, Edmonton, Alberta, Canada; 2) IWK Health Centre, Halifax, Nova Scotia, Canada; 3) North York General Hospital, Toronto, Ontario, Canada.

Ring chromosome 14 is associated with typical clinical features including prenatal and postnatal growth retardation, mental retardation, characteristic facies and retinitis pigmentosa. Seizures are present in all cases. Seizures could be due to deletion of a locus in the preterminal region of 14q on one homologue. The presence of such a locus on 14p seems unlikely, as in patients with Robertsonian translocations of chromosome 14 seizures are not frequently found. Seizures seen in r(14) patients may be also due to the mitotic instability of the ring chromosome leading to mosaicism. However, seizures are not universally present with all ring chromosomes. In the present study we have used BAC probes in the distal region of chromosome 14q to characterize deletions in six patients determined cytogenetically to have a ring or terminal deletion on chromosome 14, with breakpoints at 14q32.32-32.33. We observed marked differences in the size of deletions in the patients studied. The deletions ranged from 0.6 Mb to 5 Mb. In one patient, the apparent terminal deletion was actually due to a 7.5 Mb interstitial deletion: del(14)(q32.12q32.31). There was no common region of deletion in r(14) as opposed to terminal deletions. We found exactly the same localization of breakpoints in one r(14) patient and one terminal 14q deletion patient. The phenotypes of these patients shared common features, such as microcephaly, hypotonia and global developmental delay, but the r(14) patient phenotype was more severe and included seizures and retinitis pigmentosa. Thus, it seems unlikely that there was a specific locus in 14q32.3 that predisposed to seizures or retinitis pigmentosa. The cause of these features in r(14) patients was probably the formation of the ring itself. The ring formation changes the chromatin architecture of the chromosome. Despite the size of the deletion on 14q, the juxtaposition of the DNA from q-arm near the inactive heterochromatin of the centromere and p-arm could cause silencing of adjacent genes.

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Delineation of 1p36 deletion sizes using a panel of FISH prob. *A. Adeyinka, T. Brimmer, E.C. Thorland, S.A. Adams, S.M. Jalal.* Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.

Deletion of 1p36 is a recently delineated syndrome characterized by dysmorphic features, developmental delay and psychomotor retardation. This terminal deletion is often difficult to detect cytogenetically. Among 2257 consecutive patients with idiopathic mental retardation, developmental delay and/or mild to moderate dysmorphism that were studied from 2001 to 2003 using subtelomeric FISH probes, eight patients with anomalies of 1p36 were identified. Six had terminal deletions of 1pter and two had unbalanced translocations resulting in a derivative chromosome 1 with del(1)(pter). Three of the six patients with del1pter and one of the two with unbalanced translocations had loss of the p73 probe signal, whereas the others retained the P73 locus, implying a breakpoint range of 1p36.3-1p36.1. To better delineate the deletion sizes in these patients we have applied a panel of FISH probes developed from PACS and BACs about 0.5Mb to 1Mb apart on 1p36. Preliminary findings from three patients with loss of the p73 probe and a fourth patient with a der(1)t(1;13) ascertained by G-banding revealed breakpoints ranging in size from ~ 4Mb to more than 7.5 Mb from the telomere. The difference in deletion sizes, may in part account for some of the phenotypic differences observed among patients with del(1)(p36).

Microduplication 22q11.2 syndrome: clinical and molecular cytogenetic analysis of a new case. A. Ardalan¹, F. Lebas², L. Finkel¹, V. Mucignat², S. Ducrocq², S. Marlin³, V. Malan¹, F. Gold², JL. Taillemite¹, MF. Portnoi¹. 1) Laboratoire de Cytogenetique, Hopital Saint-Antoine, 75012, Paris, France; 2) Service de Neonatologie, Hopital Armand-Trousseau, 75012, Paris, France; 3) Service de Genetique, Hopital Armand-Trousseau, 75012, Paris, France.

Recently, some patients with variable clinical phenotype, ranging from mild learning disability to the presence of severe congenital malformations or some overlapping features with DG/VCF syndromes, have been shown to harbor a chromosome duplication 22q11.2 of the genomic region that is deleted in patients with DGS/VCFS. The microduplication of 22q11.2, establishing the complementary genome disorder of the 22q11.2 deletion syndrome, appears to be a new chromosomal microduplication syndrome. The reported cases have been identified primarily by interphase FISH and could escape identification and be missed by routine cytogenetic analysis. Hence, this new syndrome might be underdiagnosed. We report on a 2-month-old girl, who presented with cleft palate, minor dysmorphic features including short palpebral fissures, widely spaced eyes, long fingers, and hearing loss. She was referred to rule out 22q11.2 microdeletion diagnosis of VCFS. Her mother had mild mental retardation, learning disabilities and similar facial features. FISH analysis of interphase cells showed three DG/VCFS critical region (N25)-probe signals with two chromosome specific identification probes in each cell. FISH analysis did not reveal the duplication on the initial testing of metaphase chromosomes, on review, the area was brighter on one chromosome 22 in each metaphase spread. Her parents were tested. The mother had the same interstitial duplication of 22q11.2. To define precisely the size and the proximal and distal breakpoints of the duplication, FISH with a panel of chromosome 22q11.2 specific BAC clones have been performed. This case illustrates the importance of scanning interphase nuclei when performing FISH analysis for any of the genomic disorders. The paucity of reported cases of 22q11.2 microduplication likely reflects a limitation in the techniques used and/or a significantly milder phenotype than do patients with deletion.

Familial complex 3q;10q rearrangement unravelled by subtelomeric FISH analysis. A. Battaglia^{1,3}, A. Novelli², C. Ceccarini², J.C. Carey³. 1) Stella Maris Clinical Research Inst for Child & Adol Neuropsychiatry, Pisa, Italy; 2) CSS Mendel Inst., Rome, Italy; 3) Division of Medical Genetics, Dept. Pediatrics, Univ. of Utah, SLC, USA.

In recent years, subtelomeric rearrangements have been identified as a major cause of MCA/MR syndromes. As of now, more than 2,500 individuals with mental retardation have been tested and subtelomeric rearrangements were detected in about 5%. Therefore, subtelomeric FISH analysis is indicated as a second tier test after high-resolution-G-banding analysis in subjects with otherwise unexplained developmental delay/mental retardation and/or MCA. We describe a female patient and her 3 maternal female cousins, all showing an undiagnosed MCA/MR syndrome, associated with the same complex subtelomeric rearrangement. Subtelomeric FISH testing performed between 3 and 18 years after the initial karyotype showed, in all 4 subjects, distal trisomy 3q and distal monosomy 10q as follows: 46,XX,ishder(10)t(3;10)(q29;q26.3)mat(D10S2488+,D10S2490-,D3S1272+,D10Z1+). BACs demonstrated that the 3q dup was 5 Mb while the 10q del was about 3.2 Mb. Parental subtelomeric FISH analysis revealed the proband's mother and her 3 of 4 brothers and 1 of 2 sisters to have a cryptic balanced 3:10 telomere translocation. The brothers with the balanced translocation were the father to the proband's cousins. All 4 affected girls showed a similar phenotype with pre/postnatal growth retardation, microcephaly, severe DD/MR, very poor/absent speech, and a distinct pattern of malformation. There were coarse face with low frontotemporal hairline, thick eyebrows, epicanthal folds, prominent/squared nose, large ears, prominent chin; brachydactyly; hypotonia. Communication was gesture mediated; graphic abilities were limited to scribbling. To our knowledge, a complex subtelomeric rearrangement as the one seen in our patients has not been reported. Monosomy 10q has been described either isolated or as part of a complex rearrangement involving telomeres other than the 3q. Trisomy 3q29 has not yet been reported, but our patients resembled cases with 3q25 trisomy suggesting that the critical region of duplication for this phenotype is in 3q29.

A Complex Chromosomal Rearrangement associated with Mowat-Wilson Syndrome. *E.L. Engenheiro^{1,3}, M. Pinto², G. Soares², I. Marques³, V. Kalscheuer⁴, N. Tommerup¹*. 1) Wilhelm Johannsen Centre for Functional Genome Research, Institute of Medical Biochemistry and Genetics, University of Copenhagen, Blegdamsvej 3, 2200 Copenhagen N; 2) Instituto de Genética Médica Jacinto Magalhães, Porto, Portugal; 3) Unidade de Citogenética, Faculdade de Medicina da Universidade de Coimbra, Portugal; 4) Max Planck Institute for Molecular Genetics, Berlin, Germany.

We report the first case of a complex chromosomal rearrangement (CCR) associated with Mowat-Wilson Syndrome (MWS). The CCR is a de novo $t(2;12;18)(q22.3; 12q22; q21.33)$ in a 19 year old female whose phenotype includes severe mental retardation, agenesis of corpus callosum, myopia, microcephaly, epilepsy and cerebral atrophy. Part of this phenotype, except for myopia, is equal to Mowat-Wilson Syndrome (MWS) (OMIM #235730), an autosomal dominant complex developmental disorder, which is known to be caused by heterozygous deletions or truncating mutations of the *ZFX1B* homeobox zinc finger gene. The breakpoint on chromosome 2 has been mapped by Fluorescence In Situ Hybridisation (FISH) using bacterial artificial chromosomes (BACs) to a ~200 kb region between RP11-95o9 and RP11-294g19 which includes the 5-end of *ZFX1B* and a cluster of recently discovered ultraconserved elements (UCs). These UCs which are 100% conserved in human, rat and mouse genomes, are located in the vicinity or overlapping with genes that are thought to be essential for the ontogeny of mammals and other vertebrates (Bejerano et al. 2004). The 12q22 breakpoint is within a ~600 kb region flanked by BACs RP11-3k15 and RP11-490g8 that contains 5 annotated genes, and the 18q21 breakpoint is within a cluster of Serpin B genes. Except for myopia, the phenotype is entirely compatible with MWS caused by truncation or disturbance of *ZFX1B*. This first CCR associated with MWS illustrate that CCRs may be associated with monogenic disorders. Furthermore, the CCR includes a breakpoint on chromosome 18q21, which has been referred to as a possible hotspot for CCR occurrence (Batanian and Eswara 1998).

Pericentric inversion, inv(14)(p11.2q22.3), associated with Goldenhar syndrome. *J.C. Hawkins, J.K. Northup, D. Matalon, R. Matalon, L.H. Lockhart, G.V.N. Velagaleti.* Dept. Pediatrics, University of Texas Medical Branch, Galveston, TX.

Goldenhar syndrome, also called hemifacial microsomia (HFM)(MIM 164210) is a birth defect involving 1st and 2nd branchial arch derivatives with an incidence of 1/5000. The variable phenotype includes mostly unilateral deformity of the external ear and small ipsilateral half of the face with epibulbar dermoid and vertebral anomalies, prompting the designation oculoauriculovertebral dysplasia (Gorlin et al., 1963). Most cases are sporadic, though autosomal recessive and autosomal dominant inheritance have been proposed. A Genome wide search for linkage in 1 family suggested linkage to a region of 10.7 cM on chromosome 14q32, between the microsatellite markers D14S987 and D14S65. Analysis of the candidate gooseoid gene within this region showed no mutations in several sporadic cases (Kelberman et al., 2001). We report an 8 month-old, Hispanic male referred for genetic evaluation. He is the 2nd child of a 33 year-old, G7P2A5 diabetic mother. On physical examination, his height (66 cm), weight (6.7 kg) and OFC (42.5 cm) were all below the 5th percentile. He has an asymmetric face, micrognathia and a crumpled ear. He has a complex cardiac anomaly including ASD, VSD and PDA. A Skeletal survey showed butterfly vertebra in T7 and hemivertebra in T9. There was also absence of epiphyseal ossification in the proximal humeri, distal femur and proximal tibia. Based on the phenotypic features, HFM was diagnosed. Chromosome analysis from cultured peripheral blood lymphocytes showed a pericentric inversion of chromosome 14, with the karyotype 46,XY,inv(14)(p11.2q22.3). FISH analysis with BAC probes from 14q confirmed the inversion and also assigned the distal breakpoint to q22.3. Preliminary analysis of MIM databases showed that the bone morphogenetic protein BMP4 is localized to 14q22-q23 region. BMP4 is a vital regulatory molecule that functions through out the development. In mouse Bmp4 is found to be essential for proper atrioventricular septation. Further FISH analysis with BAC probes localized to the 14q22.3 region is in progress to map the breakpoint and to see what role BMP4 has in HFM.

Variable presentations of 11q- deletion disorder (Jacobsen Syndrome). *M. Hummel¹, F. Keller¹, P. Grossfeld², JE. Coad¹, SL. Wenger¹.* 1) West Virginia Univ, Morgantown, WV; 2) University of California, San Diego, CA.

11q- deletion disorder (previously called Jacobsen syndrome) is a chromosome deletion disorder involving the long arm of chromosome 11, and is characterized by psychomotor retardation, cardiac defects, blood dyscrasias and craniofacial anomalies. The incidence is estimated to be 1 in 50,000. Most cases arise from either a de novo deletion or an unbalanced segregation from a balanced carrier. We describe 3 new cases of 11q- each with different presentations. Case 1: 18 mo female who presented with developmental delays, facial dysmorphism and low platelets. Thrombocytopenia initially was attributed to alloimmune based on the presence of maternal antiplatelet antibodies. Platelet counts improved but have never normalized. Facial dysmorphism included downslanted palpebral fissures, ptosis and broad high forehead. Chromosome analysis was 46,XX,del(11)(q23.3). Parental studies were normal. Molecular studies revealed the retention of probes L7-17 and D11S1299 characterizing this as a small to moderate deletion. Case 2: 26 yo woman seen for abnormal fetal karyotype. Ultrasound showed IUGR, unilateral hydronephrosis, and clinodactyly. Amnio found a fetal karyotype of 46,XX,del(11)(q23.3). Parental studies were normal. No abnormalities were found on fetal echocardiography. Case 3: A newborn female presented with CHD, dysmorphic features and low platelets. Cardiac defect was a DORV. Clinically the patient had ptosis and hypertelorism with broad nasal bridge. Bone marrow biopsy to evaluate persistent thrombocytopenia (30k-60k) showed atypical megakaryocytes, suggestive of Paris-Trousseau syndrome. Chromosome analysis 46,XX,del(11q)(q23.3q24.4) (11q telomere FISH probe present.) Parental studies were normal. FISH mapping demonstrated retention of subtelomeric probes as well as of probes located centromerically at ~8 megabase intervals, consistent with a small interstitial deletion. Each case was ascertained through a different presentation. Platelet defects in combination with delays prompted consideration of 11q- deletion disorder in the pediatric cases. The prenatal case shows deletions of 11q should be included in the evaluation of the fetus with anomalies.

Shorter Telomeres in Older Individuals with Down Syndrome and Dementia. *E.C. Jenkins¹, M.T. Velinov¹, L. Ye¹, S. Li¹, E.C. Jenkins Jr.¹, H. Gu¹, W.B. Zigman¹, N. Schupf^{2, 1}, S. Sklower Brooks¹, D. Pang¹, W.P. Silverman¹.* 1) New York State Institute for Basic Research in Developmental Disabilities, Staten Island; 2) Gertrude H. Sergievsky Center, Columbia University, New York, New York.

Chromosome ends or telomeres are specialized structures consisting of highly conserved TTAGGG repeats that become shorter with every cell division while protecting the important coding sequences from damage during cell proliferation. A gradual loss of telomeric DNA in dividing somatic cells can contribute to replicative senescence, apoptosis, or neoplastic transformation with age. The association between telomere shortening and in vitro cellular senescence has been well established (Proc Natl Acad Sci USA 1992;89:10114-8). Information is also accumulating relative to in vivo cellular aging and telomere shortening (J Am Geriatr Soc 2001;49(8):1105-9). Since telomere shortening has been recently associated with Alzheimer's disease (AD) status in the general population (Neurobiol Aging 2003;24:77-84), we hypothesized that a similar association might exist for adults with Down syndrome (DS), a population at increased risk for accelerated aging and AD. Individuals with DS also have decreased life expectancy and have been shown to have shorter telomeres than controls. We used quantitative telomere protein nucleic acid FISH analysis of metaphase and interphase preparations to compare telomere length from six trisomy 21 individuals with dementia and their age- and sex-matched peers without dementia (all 12 individuals had Down syndrome). All analyses were conducted blind to dementia status. We observed shorter telomeres in the demented individual in all of the six pairs studied ($p=0.028$). From this preliminary study, we conclude that telomere shortening is associated with dementia in trisomy 21 and suggest that additional research may show that telomere shortening could be an indicator of dementia status. This work was supported in part by the New York State Office of Mental Retardation and Developmental Disabilities, Alzheimer's Association grant IIRG-99-1598, and by NIH grants PO1 HD35897, RO1 HD37425, and RO1 AG014673.

Large Deletion in Rett Syndrome Detected by MPLA/ CGH Analysis. *T.A. Maher¹, X.L. Huang¹, J.M. Milunsky^{1,2,3}.* 1) Center for Human Genetics; 2) Department of Pediatrics; 3) Department of Genetics and Genomics; Boston University School of Medicine, Boston, MA.

Rett Syndrome, primarily affecting females, is an X-linked disorder with a frequency of ~1 in 12,000. Typically the syndrome is a neurologic developmental disorder and one of the most common causes of female mental retardation. Molecular testing consists of sequencing the four exons of the methyl-CpG-binding protein-2 (MECP2) gene. This has been shown to detect approximately 80% of mutations in patients. The mutational spectrum consists of missense, nonsense, small deletions and complex rearrangements. Recently, a relatively new method, Multiplex Ligation-Dependent Probe Amplification (MPLA), has been applied to Rett analysis. MPLA is multiplex, quantitative PCR method to determine copy number. Probes are specific to each of the four exons and control regions outside the gene. This technique has detected whole exon deletions that were missed by sequencing.

An 18 year old female's sample was submitted for sequencing of the MECP2 gene. She has typical Rett syndrome features including autism and no speech. She remains ambulatory. After sequencing analysis of the four coding regions, no nucleotide changes were detected. Here we report a large deletion detected using the MPLA method. MPLA analysis was completed and a deletion of exon 4 was detected along with a deletion of one of the control probes some ~7Mb away. This result was confirmed using high resolution comparative genome hybridization (CGH) with the deletion estimated to be ~9Mb. Further studies continue to refine the deletion and determine if other genes may be involved. MPLA analysis is relatively straight forward to implement and will increase the mutational spectrum of Rett. We have instituted this method as part of our clinical testing protocol.

Phenotype-Genotype correlations of dicentric Y chromosomes: the third review of literature. *N. Abdelmoula¹, A. Amouri², MF. Portnoi³, A. Bahloul⁴, A. Saad⁵, T. Rebai¹.* 1) Lab d'Histologie, Fac de Medicine de Sfax, Tunisia; 2) Lab de cytogenetique, Inst Pasteur de Tunis, Tunisia; 3) Lab de cytogenetique, Hopital Saint-Antoine, Paris, France; 4) Service d'Urologie, Hopital Habib Bourguiba, Sfax, Tunisia; 5) Lab de cytogenetique, Hopital Farhat Hached, Sousse, Tunisia.

Dicentric Y chromosomes have been reviewed twice in 1995 by Hsu LYF. and Tuck-Muller C. who showed that dic(Y) are the most common Y structural abnormalities and that their influence on gonadal and somatic development is extremely variable. Here, we report a third comprehensive review of the literature concerning dicentric Y chromosomes reported since 1994. We find 78 new cases for which molecular studies (PCR or FISH) have been widely applied to investigate SRY (68% of cases), GBY, ZFY, RFS4Y, GCY and different genes at AZF region. For dic(Yq), all cases (20) were mosaic for 45,X and 4 of them were also mosaic for a 46,XY cell line. When breakpoints were available (15/20 cases), they were in Yp11. 50% of cases were phenotypic female and 20% phenotypic male while 20% of cases were reported with gonadal dysgenesis. Gonadal histology was defined in 8 cases but only in one case, gonadal tissue was genetically investigated because of gonadoblastoma. For dic(Yp), mosaicism concerned only 45,X cell line and was found in 50 cases while the remainder five cases were homogeneous. When breakpoints were available, it was at Yq11 in 50 cases and at Yq12 in two cases. 54% of cases were phenotypic female, 26% were phenotypic male and 18% were associated with genitalia ambiguous. SRY was analyzed in 33 cases, sequenced in 9 cases and was muted in only one case. Gonads were histologically explored in 34 cases and genetically investigated in 8 cases. Gonadoblastoma was found in only two cases. Through this review, it seems that phenotype-genotype correlations are still not possible and that homogeneous studies of dic(Y) in more patients using molecular tools for structural characterization of the rearranged Y chromosome and assessment of mosaicism in many organs are necessary to clarify the basis of the phenotypic heterogeneity of dicentric Y chromosomes and then to help phenotypic prediction of such chromosome rearrangement.

FISH evaluation of 15q11-13 microdeletions in sperm nuclei. *E. Anton, J. Blanco, J. Egozcue, F. Vidal.* Unitat de Biologia Cel.lular (Facultat de Ciències), Universitat Autònoma de Barcelona. 08193-Bellaterra. Spain.

Most of the cases of Prader-Willi Syndrome (PWS) (70 %) are due to a paternal 15q11-13 microdeletion. The majority are considered to be originated *de novo*, as the consequence of functional meiotic errors occurring during the process of gametogenesis. With the aim of investigating if fathers of PWS children have an increased tendency to form sperm cells with *de novo* microdeletions or whether this is a random phenomenon, we have performed a fluorescence in situ hybridization (FISH) study in semen samples from 5 control men and 5 fathers of children affected by PWS. A triple-color FISH approach using probes for the centromeric region of chromosome 6 labeled in Aqua (used as a ploidy control), centromeric probe for chromosome 15 labeled in Green and a locus-specific probe for the 15q11-13 region labeled in Orange (Vysis Inc.) were used. Basal hybridization failure for the 15q11-13 probe was evaluated in lymphocytes, resulting in a 2.06%. All analyses were blindly conducted with respect to the genetic origin of the syndrome. In control donors, percentages of spermatozoa lacking an orange signal fell within the values expected from hybridization failures. Similar results were observed in the semen sample of the only father having a PWS child originated by uniparental disomy. Results in fathers of children affected by microdeletions showed a divergent pattern with an increased frequency of spermatozoa with the 15q11-13 microdeletion in one of the four cases analyzed. Although more patients should be analyzed to gather more significant data, these preliminary findings do not suggest a predetermined differential meiotic behavior in these men though some individuals might have a predisposition to produce microdeleted gametes. In this sense, FISH in sperm nuclei could be a useful tool to help in the evaluation of recurrence risks.

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Micronuclei in human lymphocytes treated with chloroquine *in vitro*. *I.P. Aranha, C.L.R. Chagas.* Inst Biol, PHLC S-525-6, Univ Estado Rio de Janeiro, Rio de Janeiro, Brazil.

Chloroquine is a drug that was initially used for the treatment of malaria but has also been successfully used for the treatment of arthritis and lupus eritematosus. In the present study, the micronucleus assay in human lymphocytes was used to assess the effects of chloroquine *in vitro*. Micronuclei appear during cell division as a result of acentric chromosome fragments or whole chromosomes, outside the nucleus. Blood was collected from healthy donors 18 to 30 years old. Peripheral whole blood cells were incubated at 37°C and 5% CO₂ for 72h, in enriched RPMI 1640 medium in the presence of chloroquine (15ng/ml). Cells not exposed to the drug served as control for the experiment. Cytochalasin B (4g/ml) was added to the cultures 44h postinitiation. After fixation, cells were stained with Giemsa Gurr (2%) and analysed under optical microscope. In the test group, from 3382 binucleated cells analysed 17 micronuclei were observed, while in the control group, from 2899 binucleated cells analysed 1 micronucleus was found. Using the chi-square test with Yates correction, the results were very significant ($p=0.0013$) showing an association of chloroquine and the occurrence of micronuclei.

Molecular and cytogenetic characterization of an idic(Y)(p11.23) chromosome in a boy with right testicular dysgenesis, left gonadism and growth retardation. A. Bahloul¹, N. B. Abdelmoula², A. Amouri³, MF. Portnoi⁴, M. Hachicha⁵, A. Saad⁶, T. Rebai¹. 1) Service d'Urologie, CHU H Bourguiba, Sfax, Tunisia; 2) Lab d'Histologie, Faculte de Medecine de Sfax, Tunisia; 3) Lab de Cytogenetique, Inst Pasteur de Tunis, Tunisia; 4) Lab de Cytogenetique, Hopital Saint-Antoine de Paris, France; 5) Service de Pediatrie, CHU H Chaker, Sfax, Tunisia; 6) Lab de Cytogenetique, CHU F Hached, Sousse, Tunisia.

We report a 15 years old boy who was referred for ambiguous genitalia and growth retardation with short stature (below the 4th percentile). His external genitalia were represented by a small phallus with urethral opening at its perineal insertion and a labioscrotal folds fused with minimal rugation. At surgery, ambiguous genitalia was corrected in male senses. No right gonad was founded and a left rudimentary gonad, appearing as a testis with epididymus and vas deferens, was removed during the operation. Histological assessment of this gonad demonstrated it to be an immature testis which contain immature seminiferous tubules with Sertoli cells but without spermatogonies. Chromosome analysis carried out from peripheral blood using RHG, GTG and CBG banding showed a 45,X cell line and an other in which the Y chromosome was replaced by an abnormal apparently metacentric chromosome. The abnormal chromosome was interpreted to be a dicentric isochromosome composed of two copies of the long arm, centromere, and proximal portion of the short arm of the Y chromosome. The cytogenetic interpretation was supported by FISH analysis using the Y heterochromatin specific probe (DYZ1), the Y alphaloid centromere specific probe (DYZ3), the X chromosome specific DNA probe (STS Xp22.3 Region probe with DXZ1 chromosome) and the LSI SRY (Yp11.3)/CEP X(DXZ1) dual color DNA probe. FISH exhibited two copies of SRY making evidence for duplication of pericentromeric sequences of Yp and corroborated a dicentric chromosome. The ratio of mosaicism was estimated by FISH analysis of 250 cells. PCR analysis using both SRY and ZFY primers were positive. The abnormal chromosome constitution in our patient was then written: 45,X[35]/46,X,dic(Y)(qter-p11.32::p11.32-qter)[65].

Multicolour banding and PCR analysis of two small derivative Y chromosomes. *S. Bal¹, M. Tomiuk¹, T. Anderson¹, D. Tucker¹, D. Riordan¹, A. Mhanni^{2,3}, A.E. Chudley^{2,3}, I. Chudoba⁴, B. Morash^{1,2,3}, A.J. Dawson^{1,2,3}.* 1) Cytogenetics Laboratory, Division of Laboratory Medicine and Pathology, Health Sciences Centre, Winnipeg, Manitoba, Canada; 2) Section of Genetics, Department of Pediatrics and Child Health, Health Sciences Centre, University of Manitoba, Winnipeg, Manitoba, Canada; 3) Department of Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, Manitoba, Canada; 4) Metasystems, Altlussheim, Germany.

A 31/2 year old male was referred to the Genetics Clinic because of speech and language delay, short stature, mild dysmorphisms and Duane anomaly of the eye. Chromosome analysis showed an unbalanced male karyotype with a modal chromosome number of 47. There was a missing Y chromosome and two different sized markers whose origin could not be determined by conventional cytogenetics. Both markers occurred de novo as both parents have normal karyotypes. FISH analysis showed a Y centromere on both markers with a larger signal present on the smaller marker, der(Y2), than on the larger marker, der(Y1). FISH analysis also showed that SRY was present on der(Y1), consistent with the proband's male phenotype, but not on der(Y2). FISH analyses with PAR1/PAR2 subtelomere probes were negative on both der(Y)s. Subtelomere FISH analysis was otherwise normal. Q-banding did not show Yqh fluorescence on either derivative. Multicolour Y chromosome banding showed that der(Y1) is derived from the Y centromere and short arm whereas der(Y2) is derived from the Y centromere and pericentromeric short and long arms. Der(Y2) had a larger centromere than der(Y1), in agreement with our previous results. PCR analysis of the spermatogenesis region of Yq11 showed that the proximal AZFa was present whereas the more distal AZFb and AZFc were deleted, likely resulting in short stature and infertility. The proband has two older brothers who also have speech and language delay but who have normal Y chromosomes. This suggests that the speech and language delay in our proband is likely coincidental to the der(Y)s, which likely accounts for the short stature and variable dysmorphisms previously reported in association with Yq deletions.

Phenotypic implications of infrastructural rearrangements of the Y chromosome: investigation by FISH using

BACs. *M. Beaulieu Bergeron*^{1,4}, *M. DesGroseillier*^{1,4}, *R. Fetni*⁵, *P. Brochu*³, *E. Lemyre*^{2,4}, *N. Lemieux*^{1,3,5}. 1) Département de Pathologie et biologie cellulaire, Université de Montréal, Montréal, Québec, Canada; 2) Département de Pédiatrie; 3) Département de Pathologie and; 4) Centre de recherche de l'Hôpital Sainte-Justine, Montréal, Québec, Canada; 5) Department of Pathology, McGill University Health Center, Montreal, Quebec, Canada.

Both males and females can carry infrastructural rearrangements of the Y, which are often unstable and complex. Despite the fact that the Y chromosome contains very few known genes, abnormalities of this chromosome can cause various phenotypes, especially in females, such as Turner syndrome, sex reversal or sexual ambiguity, and gonadoblastoma. In males, less severe clinical implications include infertility and azoospermia. We characterized 15 patients with isodicentric Y chromosomes and 7 patients with sex reversal. FISH was performed using probes SRY (Yp11.3), 91H4.5 (Yp11.2), DYZ1 (Yq12), DYZ3 (Y centromere) and WCPY (whole chromosome painting of the Y). BACs (Bacterial Artificial Chromosomes) were also used in some cases in order to refine the molecular breakpoints. SRY was found to be present in 14 cases of idic(Y). The different phenotypes seen in those 14 cases might be explained by the variable proportion of the 45,X cell line. As for the 4 cases of sex reversal with a der(X), the presence of SRY was confirmed in all, generating the male phenotype of those patients. One other case of sex reversal is explained by the duplication of DAX, resulting in a female development despite the presence of SRY. Finally, we are currently unable to explain the female phenotype seen in two patients with a predominant 46,XY cell line. In conclusion, this study highlights the necessity of using BACs to refine the molecular breakpoints of infrastructural rearrangements of the Y. Indeed, these BACs will allow us to determine if a common molecular region is implicated in specific rearrangements of this chromosome. As a result, this will not only help increase the correlation between the genotype and its clinical implications, it will also improve both genetic counselling and cares offered to patients carrying such anomalies. *Supported by RMGA-FRSQ.*

Recurrent breakpoints in balanced autosomal translocations in man. *P.A. Benn¹, M.J. Benn²*. 1) Gen/Dev Biol/Human Genetics, Univ Connecticut Health Ctr, Farmington, CT; 2) Farmington High Schl, Farmington, CT.

Objective: To evaluate the distribution of chromosome breakpoints in balanced reciprocal translocations in man.

Methods: Data were extracted from North American and European collaborative studies on couples receiving prenatal diagnosis (Daniel et al AJMG 33, 14-53, 1989). Robertsonian translocations, inversions, t(11;22)(q23-q25;q11-13), and complex rearrangements were excluded. When multiple cases with identical re-arrangements were identified through a single laboratory, the carriers were assumed to be related and the translocation was counted only once. When identical re-arrangements were identified through different laboratories, the rearrangement was counted more than once. The distribution of breakpoints was compared with that expected if breakage occurred randomly. $P < 0.01$ was considered statistically significant.

Results: After exclusions, 905 translocations with 1810 breakpoints were analyzed. There were 34 instances where identical translocations were reported by two or three laboratories and 19 (56%) of these involved reports by laboratories based in different countries. Breakpoints were non-randomly distributed. 476 (26%) of the breakpoints were assigned to the 44 terminal bands with 21q22 most commonly involved (27 cases). After excluding the terminal band breakpoints, there remained significant departures from random with an excess of breakpoints assigned to chromosome 15 and a deficit for chromosomes 12 and 19. Interstitial bands 1q32 (24 cases), 4q31 (20 cases) and 13q14 (22 cases) were each involved in over 1% of all breakpoints.

Conclusion: Balanced translocation breakpoints presumably reflect regions susceptible to rearrangement, differences in the selection against rearrangements, the chromosomal locations of essential genes, and possible ascertainment bias. These factors result in a non-random distribution in the observed breakpoints in human translocations.

Possible association of chromosome instability to recurrent abortions. *L. Bobadilla-Morales¹, M. Cervantes-Luna¹, T. García-Cobian¹, G. Zúñiga-González², J. Corona-Rivera¹, A. Corona-Rivera¹.* 1) Lab de Genetica Humana, Univ Guadalajara, CUCS, Guadalajara, Mexico; 2) CIBO-IMSS, Guadalajara, Jal., Mexico.

Recurrent pregnancy loss, is observed in 1 to 2 % of fertile women. The pathophysiological mechanism has not been well established. On the other hand, chromosome instability consists of structural spontaneous or induced aberrations. In this work we show a non consanguineous healthy couple with recurrent abortions and no constitutive chromosome aberrations, but with two tetradial chromosomes in routine female study. The couple was of 30 and 29 ys old, male and female respectively. They showed three recurrent abortions of around 13 weeks of gestation, the first of them was diagnosed as anembrionic and the third one as molar. We performed a second evaluation to the search of chromosome instability in the couple and controls by testing three different culture conditions: gamma radiation (GR), Uv light (UvL) and mitomicyn-C (MC) in 72 hs blood cultures alternatively exposed to 1 Gy of GR 3 hs prior harvesting, UvL for 30 minutes 3 hs prior harvesting and MC (20 ng/ml) after 24 hs of set up. Chromosome analysis was done in 60 mitosis in the couple and controls. UvL and MC condition scores were not different to controls. GR chromosome damage was of 22% versus 2% in controls and statistically significant only in the female who exhibited 23% of chromatid breakages, 14% of chromatid gaps, 31% of chromosome gaps, 9 % of fragments as well as 3 tetradials and one triradial. Additionally, exfoliated buccal cell micronuclei resulted moderately increased in the female. Then, chromosome instability evidences were observed in the female. Two previous reports found increased micronuclei and spontaneous breakage or aphidicolin induced fragile sites in couples with more than two spontaneous abortions, proposing an etiologic relation. In this case, the presence of spontaneous tri and tetradials and increased GR induced damage resembles instability behavior of Ataxia-Telangectasia and supports chromosome instability in the female. We propose that chromosome instability can be involved in some, otherwise cryptic, repetitive abortion cases.

Karyotypic discrepancy between the amniocytes and CVS in prenatal diagnosis and the skin tissue of the abortues. *J. Bourgeois, J. Xu.* Pathology and Molecular Medicine and Lab Medicine, Hamilton Health Sciences and McMaster University, Hamilton, ON, Canada.

A 41 year old G2P1 woman was seen in the prenatal diagnosis clinic for advanced maternal age at 14.5 weeks gestation. The routine dating ultrasound performed during her assessment was abnormal with a cystic hygroma and hydrops being noted. Counseling and chorionic villous sampling (CVS) were performed on that same day. Only 4 metaphase cells were obtained from this small (5mg) CVS sample and all had 47,XY,+13. A follow-up anatomic 18.0 week ultrasound was performed which showed resolution of the hydrops but with some persistence of the cystic hygroma. Confirmatory amniocentesis was done that same day. Prenatal interphase FISH of uncultured amniocytes using a set of the probes for chromosomes X, Y, 13, 18 and 21 resulted in 96% (48/50) of the nuclei having additional signal indicative of trisomy 13. Routine G-banding analysis of 12 in situ colonies from 4 coverslips all had 47,XY,+13. Following additional counseling regarding these results, the patient was admitted for pregnancy discontinuation with induction of labor at 19+ weeks gestational age. A single male fetus with phenotypic features consistent with a chromosomal anomaly such as trisomy 13 was delivered. Fetal skin was obtained for karyotyping and of the 10 cells, all showed trisomy 13 as well as a pericentric inversion of chromosome 5; 47,XY,inv(5)(p13q35),+13. There was no evidence of laboratory errors that might cause this discrepancy. Although parental karyotyping is being perused, this type of pre and postnatal karyotype result discrepancy is extremely rare and the exact reason for this remains unknown. A possible explanation would be the presence of isolated fetal mosaicism involving at least the fetal skin tissue that was not present in the chorionic villi or amniocytes.

Molecular mapping and phenotype of three unrelated subjects with a novel deletion 5q14.3q15. A. Boys¹, J. Ryan¹, I.E. Scheffer², W. Francis¹, D. Francis¹, I. Hayes¹, P. La¹, E. Northrop¹, H.R. Slater¹. 1) Victorian Clinical Genetics Services, Melbourne, VIC, Australia; 2) Epilepsy Research Institute and Department of Medicine, University of Melbourne, and Austin & Repatriation Medical Centre, Melbourne, VIC, Australia.

Cytogenetic deletion of the region 5q14.3 to q15 and its clinical significance has not been described. We report a subtle interstitial deletion of this region in three unrelated boys referred over a period of 10 years. The three patients have a variety of clinical abnormalities, which include severe global developmental delay, severe intellectual disability, cranial and facial dysmorphism, and visual impairment. All three boys had febrile seizures, and myoclonic seizures from birth were prominent in two. The locations and sizes of the deletions have been determined using BAC clones and FISH. Subjects 1, 2, and 3 have deletions of 7Mb, 10Mb, and 6Mb respectively. The deleted region of Subject 2 encompasses the deletion of Subject 1. There is a 1Mb overlap region common to all subjects with three known genes in this common overlap, ie. CETN3, RPC32, and MASS1. Of particular interest is the partial deletion of MASS1 in Subject 1. This gene shares part of its exonic structure with another gene called VLGR1, which itself has known isoforms. Mutations within MASS1 have been described with audiogenic seizures in the mouse and in a family with febrile and afebrile seizures. MASS1 is therefore an attractive candidate gene for the epilepsy phenotype in these del (5q) patients.

***De novo* monosomy 9p24.3-pter and trisomy 17q24.3-qter characterized by microarray CGH in a fetus with an increased nuchal translucency.** S. Brisset¹, M. Benkhalifa², A. Coulomb L'Hermine³, V. Mairovitz⁴, E. Gautier⁵, G. Tachdjian¹. 1) Dept Genetics, Hosp Antoine Beclere, Clamart, France; 2) ATL R&D, Voisins le Bretonneux, France; 3) Anatomie Pathologique, Hosp Antoine Beclere, Clamart, France; 4) Gynecologie-Obstetrique, Hosp Antoine Beclere, Clamart, France; 5) Centre de Diagnostic Prenatal, American Hospital of Paris, Neuilly, France.

Increased nuchal translucency (NT) during the first trimester of pregnancy is a useful marker to detect chromosomal abnormalities. We report a prenatal case of molecular cytogenetic characterization of an abnormal derivative chromosome 9 identified through NT. Amniocentesis was performed on a 29-year-old female because of an increased NT (4.4 mm). Chromosomal analysis revealed an abnormal 46,XX,add(9)(p24.3) karyotype. FISH (fluorescence *in situ* hybridization) with a chromosome 9 painting probe confirmed the presence of an additional euchromatic material on the short arm of one chromosome 9. A subtelomeric 9p specific probe showed a terminal deletion of one 9p. However, it was not possible to resolve the origin of this additional material on 9p. Parental chromosomes were normal. The parents were informed of the poor prognosis and a termination of pregnancy was performed at 22 weeks of gestation. In order to characterize the origin of the additional material, we performed a microarray comparative genomic hybridization (microarray CGH) using a human genomic array containing 2500 clones spanning the genome at approximately 1 Mb intervals (Spectral Genomics, Houston, TX, USA). Microarray CGH confirmed the deletion of distal 9p and showed a terminal trisomy 17q. These results were confirmed by FISH analyses using the BACs clones spotted on the array. Array CGH provided accurate information as to the breakpoint regions and the size of both distal 9p deletion and terminal 17q trisomy. The fetus was therefore a carrier of a *de novo* derivative chromosome 9 arising from a t(9;17)(p24.3;q24.3) translocation and generating a monosomy 9p24.3-pter and a trisomy 17q24.3-qter. This case illustrates that microarray CGH is a powerful and sensitive technology to identify *de novo* unbalanced chromosomal abnormalities and can be applied in prenatal diagnosis.

Trisomy 13 due to $rea(13q;13q)$ is caused by $i(13q;13q)$ and not $t(13q;13q)$ in the majority of cases. *M. Bugge*^{1,2}, *C. deLozier-Blanchet*³, *M. Bak*¹, *C.A. Brandt*^{4,5}, *J.M. Hertz*⁴, *J.B. Nielsen*^{2,6}, *L. Duprez*⁷, *M.B. Petersen*^{2,8}. 1) Wilhelm Johannsen Centre for Functional Genome Research, IMBG, Panum Institute, Copenhagen, Denmark; 2) Department of Medical Genetics, John F.Kennedy Institute, Glostrup, Denmark; 3) Division of Clinical Genetics, University of Geneva, Switzerland; 4) Department of Clinical Genetics, Aarhus University Hospital, Denmark; 5) Department of Clinical Genetics, Vejle County Hospital, Denmark; 6) Department of Clinical Genetics, Rigshospitalet, Copenhagen, Denmark; 7) Department of Genetics, Erasme Hospital, Brussels, Belgium; 8) Department of Genetics, Institute of Child Health, Athens, Greece.

It has been demonstrated, that trisomy 21 caused by $rea(21q;21q)$ de novo is due to $i(21q;21q)$ and not $t(21q;21q)$ in the majority of cases. We have used 20 PCR-based DNA polymorphisms to determine, whether trisomy 13 due to $rea(13q;13q)$ de novo in six cases is caused by $t(13q;13q)$ or $i(13q;13q)$, to determine the parental origin of the rearrangements and the mechanisms of formation. The six probands were three liveborn children with clinical features characteristic of Patau syndrome and three fetuses diagnosed prenatally by amniocentesis or CVS. Five cases were isochromosomes with two identical q arms, one of maternal and four of paternal origin. One case was a Robertsonian translocation of maternal origin.

Duplication of chromosome 3p25.3-p26.2 produces features similar to Prader-Willi syndrome. *M.G. Butler, N. Kibiryeve, M. Dasouki, J.H.M. Knoll, D.C. Bittel.* Children's Mercy Hospitals and Clinics and University of Missouri-Kansas City School of Medicine, Kansas City, MO.

Prader-Willi syndrome (PWS) is the most common genetic cause of morbid obesity and results from a functional loss of paternally expressed genes from the chromosome 15q11-q13 region. PWS is associated with neonatal hypotonia, failure to thrive, developmental delay, hypogonadism and the onset of hyperphagia between the ages of 2 to 4 years. Herein, we describe an eight year old male referred for PWS. He had pervasive developmental disorder (PDD) with marked obesity and delayed speech. Chromosome 15q11q12 methylation testing and fragile X studies were normal. Chromosome analysis by GTG-banding and fluorescence *in situ* hybridization with a whole chromosome 3 paint probe suggested a duplication of 3p25.3p26.2, a finding supported by comparative genomic hybridization. At the time of examination he was 131 cm tall (50th centile), weight was 52.7 kg (> 97th centile) and head circumference was 53 cm (25th centile). The subject had rapid weight gain at 4 years of age which has continued. The duplicated region of chromosome 3p includes several genes which may contribute to obesity and PDD, most notably, ghrelin (*GHRL*) and oxytocin receptor (*OXTR*). Ghrelin is an important appetite-stimulating hormone produced by the stomach which induces adiposity. Plasma ghrelin levels are reported to be significantly higher in individuals with PWS, while oxytocin is undetectable in the hypothalamus of PWS subjects. Plasma ghrelin was 250 pg/ml in our subject. Similarly, a transporter of GABA identified in the 3p25-p26 region, *SLC6A11*, is of interest because GABA, an inhibitory neurotransmitter, is increased in individuals with PWS and may be involved with multiple neurodevelopmental aspects of PWS. Interestingly, the *PPARG* gene is adjacent to the duplicated region of our subject. Defects in *PPARG* can lead to type 2 diabetes and predisposition to obesity. The expression levels of these genes and others located in 3p25-p26 are under investigation to determine if overexpression as a consequence of the duplication contributes to the physical and behavioral problems seen in our subject.

Determination of the origin and mechanisms of formation of three constitutive structural aberrations of chromosome 5. *A. Cervantes, N. Monroy, E. Galvez, M.C. Chima, R. Alvarez, L. Camberos, S. Kofman.* Genetica, Hospital General Mexico, F. Medicina. UNAM, Mexico DF, Mexico.

We investigate the parental origin and delimited the breakpoints in three different deletions of chromosome 5 using microsatellite analysis. The karyotypes with GTG bands of the patients were: 46,XY,del(5)(p13.3); 46,XY,del(5)(p15.3?) and 45,XX,del(5)(q22q31),der(13;14)(q10;q10). The karyotypes of mother of case 1, both parents of case 2, and the father of the last one were normal, while her mother was carrier of the robertsonian translocation. The father of case 1 was not studied. PCR amplification of genomic DNA of all of them was done using CA/GT microsatellites for chromosome 5 from the ABI-PRISM Linkage Mapping Set ver 2.0-PE and the products analyzed on an ABI310 automated sequencer. The results showed the lost of paternal alleles in the three cases. The breakpoints were assigned between D5S418 and D5S426 in case 1, confirming the terminal deletion from 5p13.1. The paternal alleles of D5S418(5p13.1) and D5S407(5q11.2) were absent in case 2 showing two interstitial deletions and the presence of 5 pairs of alleles on 5p15.3. In the girl, the deleted segment was between D5S2027 and D5S436. It has been proposed that structural aberrations occur by non-allelic homologous recombination on specific susceptible sites, originated by the genome architecture due to the presence of segmental duplications or low copy repeats, LCRs. The investigation for reported LCRs, as odorant receptors, was negative in the cytogenetic breakpoints; but some duplicons are located in 5p13, 5p14 and 5q21, additionally these regions have been implicated in other constitutive or acquired abnormalities and are rich in coding genes, LINE and SINE elements that could be involved in the rearrangements.

A translocation t(6;7)(p21;q22) associated with autism and mental retardation: localization, and identification of candidate genes at the breakpoints. *S. Choufani¹, J. Vincent^{1,3}, J. Skaug¹, W. Roberts², P. Szatmari⁴, J.M. Friedman⁵, S.W. Scherer¹.*

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Autism is a severe neurodevelopmental disorder characterized by impairments in social interaction and communication skills, coupled with unusual interest patterns and stereotyped behaviors. Genetic linkage evidence for an autism susceptibility locus (or loci) points to several regions of the genome, most notably a 50 Mb interval at 7q21 to 7q35. Supporting evidence for an autism susceptibility locus on 7q has come from cytogenetic studies of autism patients with chromosomal abnormalities. We describe here a male proband with mental retardation and autism who has a balanced translocation - t(6;7)(p21;q22). This translocation was inherited from an apparently normal father. Using fluorescence in situ hybridization, we have localized the breakpoints on both chromosomes and have identified - nearby candidate genes. These include the neural pentraxin 2 gene (NPTX2) on 7q22 and human homologue of the mouse dystonin gene (BPAG1) on 6p22. It is conceivable that either of the two breakpoints could define a new locus for autism and/or a form of mental retardation. NPTX2 is thought to be involved in excitatory synaptogenesis and could, therefore, be considered as a functional candidate for autism. In order to examine whether a genetic variation of this gene is involved in autism, we screened the 5 exons and flanking intronic sequence of the human NPTX2 gene, using direct sequencing, in 69 unrelated individuals with autism from multiplex families. We identified a total of 6 new variants, 3 coding and 3 intronic variants. We are currently testing the presence of each variant in control samples.

Genomic microarray facilitated identification of a der(4)t(4;11)(p16.3;p15.5) in a patient with a modified Wolf-Hirschhorn syndrome phenotype. *B.C. Cowley, D.A. Stevenson, J.C. Carey, P. Bayrak-Toydemir, R. Mao, A.R. Brothman.* Pediatrics, University of Utah, Salt Lake City, UT.

Wolf-Hirschhorn syndrome (WHS) is a 4p microdeletion syndrome sometimes not detectable by high resolution cytogenetics. We report a *de novo* cryptic 11p duplication found by genomic microarray with a cytogenetically detected 4p deletion. This patient, daughter of phenotypically normal parents, presented with physical features including hypertelorism, hypotonia, and multiple minor anomalies. Initial cytogenetic analysis revealed a 46,XX,del(4)(p16.3) karyotype at the 550-band level and the WHS region was confirmed to be deleted by FISH. Additional genetic abnormalities were suspected based on unusual features, including a large facial hemangioma and a full-cheeked robust appearance to her face. Her growth was atypical with normal prenatal and postnatal growth parameters; she had persistent hypoglycemia in the neonatal period, and she lacked the typical severe feeding difficulties seen in WHS. High-resolution dye-reversal genomic microarray analysis using a 2-4 Mb Human BAC chip (Spectral Genomics, Houston, TX) was performed. The 4p deletion was confirmed and its breakpoints further defined (the most proximal clone showing deletion was RP11-91B20), and an 11p duplication was also observed (the most proximal clone showing duplication was RP11-371C18). FISH using subtelomeric probes (Vysis, Downers Grove, IL) confirmed these abnormalities. The revised karyotype is thus 46,XX,der(4)t(4;11)(p16.3;p15.5). Parental chromosome analysis and FISH showed no abnormalities. Molecular short tandem repeat markers (D11S2071 and D11S4177) narrowed the breakpoint for the 11p duplication between 1.3 to 1.4 Mb from the telomere. In addition, marker D11S2071 demonstrated the duplication is of paternal origin. The 11p gain is thus hypothesized to be the cause of the modified WHS phenotype. The resolution of genomic microarray facilitated detection of an otherwise cryptic abnormality. This case is evidence for the value of genomic microarrays in clinical settings and their utility in precisely defining the genetic component behind unusual or ill-defined abnormalities.

DELINEATION OF THE CRITICAL REGION FOR GENITOURINARY ABNORMALITIES WITHIN DELETION 10q26.3. *C.A. Curtis, A.B. Zinn, S. Schwartz.* Center for Human Genetics, University Hospitals of Cleveland and Department of Genetics, Case Western Reserve University, Cleveland, OH.

A 5-day-old newborn male was referred for chromosomal analysis when he was found to have bilateral single palmar creases, widely-spaced nipples and choking spells. Cytogenetic studies revealed an unbalanced male karyotype with 45 chromosomes, including a derivative chromosome 10 that resulted from a translocation between the distal long arm of chromosome 10 (breakpoint in 10q26.3) and the proximal long arm of chromosome 22 (breakpoint in 22q11.21). Parental karyotypes were normal. Preliminary FISH studies confirmed that the derivative was deleted for the 10q subtelomere and the chromosome 22 centromere but retained the TUPLE1 locus of 22q11.21. The patient's physical examination was normal, including his genitalia, except for several minor craniofacial dysmorphic features. ECHO revealed a bicuspid aortic valve. A renal ultrasound and a voiding cystourethrogram were normal. Based on data from the Human Genome Browser (July 2003 freeze), the deleted region of 22q comprises a maximum of 1.3Mb. Additional BAC FISH studies revealed that the 10q deletion breakpoint was between RP11-941B15(+) and RP11-1141L20(-), indicating that the chromosome 10 deletion involved the terminal 3Mb of 10q. Ogata et al (2000) suggested that gene(s) involved in renal tract development and male sex differentiation are located within the 10q26 region. They estimated that the gene(s) responsible for the genital anomalies lies within the terminal 4.5Mb of 10q, distal to D10S1248, whereas the gene(s) responsible for the renal anomalies lies within the terminal 6.8Mb segment, distal to D10S186. The absence of both genital and renal malformations in our patient narrows the region associated with genital defects to ~1.65Mb, between D10S1248 and RP11-1141L28, and narrows the region associated with renal anomalies to ~4Mb, between D10S186 and RP11-1141L28. This case suggests that the most telomeric 3Mb region of 10q does not include the critical regions for either the urinary or genital abnormalities observed in patients with terminal deletions of 10q.

Real-time quantitative PCR (Q-PCR), Fluorescence In Situ Hybridization (FISH) and microarray based Comparative Genomic Hybridization (array-CGH) for the detection of submicroscopic chromosomal deletions in patients with features of holoprosencephaly spectrum. V. David¹, C. Bendavid¹, I. Gicquel¹, C. Henry², MR. Durou³, S. Odent⁴, C. Dubourg¹. 1) Fac de Medicine, UMR 6061 CNRS, Rennes, Cedex, France; 2) CHU Pontchaillou, Laboratoire de Cytogenetique, Rennes, France; 3) CHU Pontchaillou, Laboratoire de Genetique Moleculaire, Rennes, France; 4) CHU Hopital Sud, Unite de Genetique Medicale, Rennes, France.

Holoprosencephaly (HPE; 1/16000 live births; 1/250 conceptuses) is the most frequent congenital malformation of the brain. The clinical spectrum ranges from alobar HPE to semilobar and lobar HPE with accompanying facial anomalies. HPE can be due to environmental factors, major chromosomal abnormalities, polymalformative syndromes or be isolated. Ten genes (SHH, ZIC2, SIX3, TGIF, PATCHED, TDGF1/CRIPTO, GLI2, FAST1, DISPATCHED, HNF3) have been implicated in HPE but point mutations in these genes are responsible for less than 20 per cent of HPE cases. In the 80 per cent remaining, we suspect either other genetic alterations and particularly large deletions in the known HPE genes, or the implication of other genes not yet identified. This led us to use three different approaches to detect microdeletions. First we are investigating specific genes involved in HPE. We are developing real-time quantitative PCR (Q-PCR) applied to TGIF, SHH, ZIC2, SIX3 and GLI2, using SYBRGreen and TaqMan technologies for 200 newborns and 70 fetuses with HPE. We are also testing 40 lymphoblastoid cell lines using multicolour FISH targeting six genes (TGIF, SHH, ZIC2, SIX3, DISPATCHED and HNF3). Concurrently we initiated a comparative genomic hybridization (CGH) array approach to identify microdeleted regions all over the genome, using 2500 BAC arrays (Spectral Genomics). We already found a microdeletion in TGIF by Q-PCR on a fetus DNA sample, and we refined the size of this deletion to 10 Mb on chromosome 18p by the CGH array. We will present our results of Q-PCR and FISH applied to SHH, ZIC2, SIX3, TGIF, GLI2, DISPATCHED and HNF3, and our preliminary results of CGH array.

Stable ring chromosome 6 due to a centromeric fission. *M. de Blois¹, D. Sanlaville¹, A. Bachelot², C. Ozilou¹, S. Chevallier¹, M.C. Waill-Perrier¹, S. Romana¹, M. Vekemans¹, C. Turleau¹.* 1) Cytogenetics laboratory, Hopital Necker Enfants Malades, Paris, France; 2) Service of endocrinology, Hopital Necker Enfants Malades, Paris,.

A 43 year-old man was referred to us because of azoospermia. Peripheral blood lymphocytes chromosome analysis using GTG and RHG banding techniques, revealed a structurally abnormal chromosome 6p and an extra ring chromosome. FISH studies using chromosome 6 specific painting and subtelomeric probes showed respectively that the ring chromosome originated from chromosome 6 and that the 6p subtelomeric sequences were present on both the normal and the abnormally short chromosome 6. The absence of MCA/RM suggested that both structurally abnormal chromosomes 6 were totally stable. Using CBG banding technique, a signal was observed on the ring chromosome 6 but no signal was observed on the structurally abnormal chromosome 6. However further FISH studies using a chromosome 6 specific satellite probe showed a signal on both abnormal chromosomes 6. Presumably both structurally abnormal chromosomes resulted from an interstitial deletion of the short arm of chromosome 6 and a centromeric fission. Therefore we interpreted the karyotype as 47,XY,del(6)(qterp10::p22.3pter),+r(6)(p10p22.3) and the azoospermia as resulting from meiotic disturbance due to an abnormal synapsis of chromosome 6 homologues. Several observations of interstitial deletions associated with a ring chromosome have been reported in the literature. A neocentromere is observed either on the ring chromosome or on the deleted chromosome. As in the present observation satellite sequences were present on both the deleted chromosome and the ring chromosome, we concluded that its mitotic stability did not result from the presence of a neocentromere. It is well known that centromere fission played an important role during evolution but so far only a small number of cases have been reported in humans.

Array-CGH (aCGH) characterization of a distal 12q duplication in a child with mild language delay and partial epilepsy. *M. Demos*¹, *B.P. Coe*², *J.C. Wang*³, *B. Lomax*³, *M. Connolly*⁴, *W.L. Lam*², *P. Eydoux*³. 1) Medical Genetics, Children's and Women's Hosp; 2) B.C. Cancer Agency; 3) Cytogenetics Laboratory, Children's and Women's Hosp; 4) Pediatric Neurology, Children's and Women's Hosp; Vancouver, B.C., Canada.

Chromosomal abnormalities associated with epilepsy include terminal deletion of chromosome 1q and the inverted duplication chromosome 15 syndrome. To our knowledge, distal rearrangements of chromosome 12q have not been considered to have a high association with epilepsy. We describe a 3 year old girl with epilepsy and mild language delay due to a chromosome rearrangement at 12q24. Seizures began at 2 years of age with fever then re-occurred with or without fever. They were complex partial or secondarily generalized. Examination revealed head circumference above the 98th percentile and mild generalized hypotonia. Head CT scan was normal and EEG showed bifrontal dysrhythmia. Her karyotype revealed additional material on the terminal region of chromosome 12q, interpreted as a duplication of 12q24. A similar rearrangement was present as a mosaic in the mother. Duplication of chromosome 12q24 was confirmed using a subtelomeric 12q probe. Using a Sub-Megabase Resolution Tiling-set (SMRT) array-CGH (aCGH), we were able to confirm the duplication of chromosome 12q24, and narrow down the duplicated region to 12q24.32-q24.33. Further, we identified a potentially deleted region in the proximal end of band 12q24.32. Our aCGH data will be confirmed by FISH using BAC probes. To our knowledge, only 4 cases of pure duplication 12q24-qter have been reported. In our patient, epilepsy may have resulted from duplication of region 12q24.32-qter, or from the potentially deleted region 12q24.32. Further characterization of our patients imbalance may allow the identification of new gene(s) associated with epilepsy. Our results illustrate the power of aCGH for the characterization of chromosomal rearrangements, allowing the detection of imbalances overlooked by conventional cytogenetics. We believe that aCGH data may be necessary for the interpretation of clinical data associated with chromosomal anomalies.

Distal 2q deletion and 10p duplication detected by genomic microarray in a dysmorphic globally delayed child.

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We report a 21-month-old Caucasian female with dysmorphic features, abnormal brain MRI, and global developmental delay. Patient ES was the 8 lb 1 oz, 21.5 inch product of a 39-week pregnancy delivered via caesarean section to a G5 P2 SA 3 28 year old female. The pregnancy was uncomplicated and there was no report of in utero exposures. On physical exam, height was at the 75-90th centile, weight at the 25-50th centile, and OFC at the 25-50th centile. Notable facial features include short, upward slanting palpebral fissures with Brushfield spots, prominent forehead, sparse hair, midface hypoplasia, beaked nose with a short columella, long philtrum, and a small mouth with down-turned corners. She has wide-spaced nipples, normal genitalia, and generalized hypotonia. She was not sitting or walking and had less than 10 single words at 16 months of age. Brain MRI demonstrated decreased bone mass and enlarged ventricles. Family history is significant for 2 spontaneous abortions in the mother, two paternal uncles with severe mental retardation and no reported birth defects, four additional paternal aunts and uncles who are alive and well with normal intelligence, and a single spontaneous abortion in the paternal grandmother. Parents are non-consanguineous. High resolution chromosome studies demonstrated a 46, XX, del(2)(q37.3) karyotype. The deletion was further characterized by genomic microarray analysis using the Spectral Genomics (Houston, TX) 1 Mb platform. Genomic microarray indicated a deletion from 2q37.1 to the telomere and duplication of 10p15.1 to the telomere. The corrected karyotype was thus 46, XX, der(2)t(2:10)(q37;p15) indicating monosomy of distal 2q and partial trisomy of distal 10p in this patient. FISH analysis with 2q and 10p subtelomere probes confirmed this finding. Parental studies are pending. This case indicates the power of genomic microarray analysis for rapid and thorough characterization of subtle chromosomal abnormalities. Its clinical use will better characterize chromosome disorders resulting in an immediate impact on genetic counseling for families.

Molecular characterization of breakpoints of an X-Y translocation in a girl with sex reversal. M.

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We report the case of a female infant born after a normal pregnancy and delivery whose high resolution karyotype was 46,X,der(Y)t(X;Y)(p21.1;q11.23). She presented at 9 d with weight loss and respiratory distress, and physical exam showed facial dysmorphism, female external genitalia and marked hypotonia; an ASD (type II) and gastro-oesophageal reflux were subsequently documented. The presence of Mullerian structures (uterus and Fallopian tubes) was described at the time of gonadectomy (age 1.5 y). Dysgenetic gonads containing no germinal cells were noted on histological examination, as was the presence of bilateral vas deferens and rete testis in addition to normally-developed Fallopian tubes. Her infancy was characterized by intermittent failure to thrive due to multiple hospitalizations for respiratory infections and a chronic pneumopathy, but height and weight have been maintained at the 25th-50th centile with gavage feeding. Neurological evaluation at age 4 revealed global developmental delay, persistent central hypotonia, multifocal myoclonia and MRI findings including gyral simplification, a hypoplastic corpus callosum and periventricular loss of white matter suggestive of abnormal cellular migration. The GTG-banded karyotype showed an SRY+ Y chromosome, with additional X chromosomal material in terminal Y revealed by FISH probes WCPY and WCPX. The breakpoint in Yq occurred between BAC clones RP11-1077O23 and RP11-933N2, both located in Yq11.23, whereas the breakpoint in Xp occurred between markers DXS1068, located in Xp11.4, and DXS538, located in Xp21.1. Partial disomy of Xp genes including DAX1, explains the gonadal phenotype of the patient, as has been reported previously. Her neurological prognosis remains reserved, as has also been reported in other XY females with extensive disomy for X chromosome genes (This research was supported by grant from Réseau de Médecine Génétique Appliquée-FRSQ).

Array-CGH: a new tool for the characterization of constitutional structural cytogenetic abnormalities. *P.*

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Genome imbalances are a major cause of cancer, developmental delay and congenital anomalies. Techniques for the study of genomic diseases include karyotyping and Comparative Genomic Hybridization (CGH), a DNA-based technique first developed using differentially labeled test and control DNA hybridized on target metaphase chromosomes. Array-CGH (aCGH) has been recently developed, where the target is no longer metaphase chromosomes but DNA spotted on slides. aCGH was very successful in identifying new regions of imbalances (deletions and amplifications) and new genes involved in cancer genesis or progression. aCGH has also been used for the detection of common microdeletions. A few studies of patients with mental retardation yielded promising results using 1-3Mb resolution arrays, identifying genome imbalances potentially responsible for the phenotype. Using a tiling resolution array consisting of 32433 overlapping BAC clones covering the entire human genome (SubMegabase Resolution Tiling set: SMRT array), we studied patients with chromosomal abnormalities including apparently balanced translocations, unbalanced translocations, deletions and duplications. This technique can be implemented in a clinical setting. In a one-step experiment, SMRT-aCGH precisely identified the regions of imbalance. Some of these regions were overlooked by conventional cytogenetics. These results were confirmed by FISH using BACs chosen according to the aCGH data. Therefore, identification of the breakpoint regions was greatly facilitated by our aCGH results and gave new insight on the type of chromosomal rearrangements. Using the Human Genome data, this powerful approach allows identifying genes which may have a role in the phenotype of structural chromosomal anomalies and also facilitates the study of the genomic structure at the breakpoints and its role in the occurrence of chromosomal rearrangements. We believe that aCGH is a useful adjunct to cytogenetic studies and must be performed prior to attempting phenotype-genotype correlations.

Disruption of Friend of Gata 2 gene (FOG-2) by balanced chromosomal translocation t (8;10) is associated with endocrinological and heart defects. *P. Finelli^{1,2}, M.T. Bonati¹, D. Giardino¹, S. Russo¹, M.P. Recalcati¹, E. Valtorta¹, D. Colombo¹, A.I. Pincelli³, F. Cavagnini³, L. Larizza^{1,2}.* 1) Laboratory of Medical Genetics Istituto Auxologico Italiano, Milan; 2) Department of Biology and Genetics, University of Milan; 3) Ospedale San Luca, Istituto Auxologico Italiano, Milan.

We report on a 11 years old male with early puberty, high levels of blood FSH and LH, bilateral gynecomastia and advanced bone age. An interatrial septum heart defect was identified at birth. Laboratory investigation started with karyotypic analysis revealing the presence of a de novo balanced translocation 46,XX,t(8;10)(q24.1;q22.1). In order to precisely define the location of translocation breakpoints we performed FISH analyses with BAC clones mapping to the chromosomal bands 8q22-q24 and 10q21-q22. We mapped the translocation bkps to 8q23.1 and 10q21.1 by FISH results given by the encompassing BACs 161E14 and 269I2, respectively. Fine mapping of the rearrangement demonstrated that the 8q bkp falls within the Friend of GATA protein 2 gene (FOG-2) located in 8q23.1 and disrupts it, whether the 10q bkp does not fall within a coding sequence. The zinc finger protein encoded by FOG-2 is a widely expressed member of the FOG family of transcription factors. The FOG proteins modulate the activity of proteins belonging to the GATA family, which are key regulators of hematopoiesis and cardiogenesis and play also a role in early gonadal development and sexual differentiation in mammals. It has been demonstrated that the FOG-2 protein can both activate and down-regulate expression of GATA-target genes, suggesting different regulation of gene expression in different promoter contexts. FISH experiments also mapped the FOG-2 bkp within IVS3, a finding which predicts a truncated protein where only the first of the eight zinc finger motifs of the wild type the protein is maintained. It is presently unknown whether haploinsufficiency or the altered function of a truncated protein are responsible for the observed clinical phenotype.

Supernumerary 15q inverted duplication associated with interstitial chromosome 15q duplication. *P. Flodman, C. Modahl, L. Mays, P.A. Filipek, M.E. Bocian, M. Hanouni, M.A. Spence, M. Smith.* Dept. of Pediatrics, Univ California, Irvine, CA.

Duplications of 15q11-q13 may arise as interstitial duplications or the duplication process may lead to generation of an extra bisatellited chromosome, a 15q inverted duplication (inv.dup.(15)). Huang et al., Cook et al. (1997) determined that these duplications are only of significance if they contain the Prader Willi Angelman syndrome (PWAS) critical region and if they arise from a maternally derived chromosome.

Structural changes within chromosome 15 may predispose to development of an 15q inverted duplication chromosome during meiosis. Long et al. (1998) reported that patient with a PWAS region negative inv.dup.(15) had an interstitial triplication of proximal 15q11-q13, including the PWAS region, on one member of the chromosome 15 pair. We report results of molecular cytogenetic studies in a patient who was first noted to have behavioral problems at age 29 months. On developmental evaluation at 36 months, he exhibited stereotypical play and used fewer than 6 words. At 48 months he met ADI/ADOS criteria for autistic disorder. Overall IQ (Stanford Binet) was 92, with relative strength in abstract/visual and quantitative reasoning, and weakness in verbal reasoning. The child is somewhat obese and has cupped pinna but is not otherwise noted to be dysmorphic. The marker chromosome hybridizes only to probes located between 15 centromere and BP1. It does not hybridize to BACs from the BP1-BP2 region or to BACs from the PWAS region (BP2-BP3 region). Molecular cytogenetic analysis revealed that, in addition to the inv.dup.(15), an interstitial duplication is present on one member of the "normal" chromosome 15 pair. This interstitial duplication was detected using BACs that encompass the Necdin, Makorin, MAGEL2 and NECDIN encoding region and BACs for the SNURF SNRPN-UBE3A region. Results of our studies indicate that detailed FISH studies not only of the inverted duplication but also of the "normal" members of the chromosome 15 pair are required to determine the clinical significance of small 15q inverted duplications.

CRYPTIC SRY MOSAICISM IN A 45,X MALE. *I.K. Gadi¹, J.R. Tepperberg¹, L. Wisniewski¹, PalD. Singh-Kahlon¹, E.S. Cantu¹, C. Bullen¹, F. Nieves¹, D. Spitz².* 1) Dept Cytogenetics, Laboratory Corp of America, Res Triangle Pk, NC.27709; 2) The Cancer Institute, 1395 State Rd #7, Wellington,FL33414.

A 22 year old man with anemia and mastocytosis was referred to cytogenetics to rule out leukemia. Analysis of bone marrow unstimulated cultures showed a 45,X chromosome complement with an apparently normal G-banding pattern in all cells. No leukemic clonal chromosome anomalies were observed. Loss of the Y chromosome is generally considered a normal male aging phenomenon, although a 45,X leukemic clone was plausible with a submicroscopic alteration. It was equally plausible that the absence of the Y chromosome in a young male would reveal cryptic localization of the testis determining gene, SRY. Interphase FISH studies of the marrow showed 11% of the cells positive for a SRY hybridization signal. A few metaphases were found showing localization of SRY at the distal short arm of a G-group chromosome. Our hypothesis is that there was a rare Y-autosomal translocation during meiosis with subsequent constitutional mitotic loss of SRY from the G-group chromosome. The gonadal tissue is very likely to have a higher ratio of SRY bearing cells than the blood in order to support apparently normal male differentiation. Clinical and molecular Y chromosome DNA follow-up to confirm infertility is pending.

Low-copy repeats and monosomy 1p36. *M. Gajecka*¹, *B.C. Ballif*¹, *C.D. Glotzbach*^{1,2}, *K.A. Bailey*^{1,2}, *L.G. Shaffer*^{1,2}.
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Some chromosomal rearrangements have been shown to arise through nonallelic homologous recombination between low-copy repeats (LCRs). In investigating the molecular basis of monosomy 1p36 we observed two putative LCRs within the terminal 3.5 Mb of 1p36. Because regions of the genome that contain LCRs and segmental duplications are difficult to map and sequence, it was necessary to closely examine this region to verify its precise structure and sequence. We identified the LCRs by systematically performing BLAT searches with 10 kb segments of genomic sequence from BACs mapping to distal 1p36 against actual draft sequence of the human genome. Using monochromosomal hybrids of chromosome 1, several BAC clones from the analyzed region and fosmids spanning these BAC clones, we performed multicolor fiber FISH and PCR walking to verify the current 1p36 genomic assembly (July 2003). We identified a 66 kb paralogous sequence (LCR 1p36A) that is present in two non-overlapping BACs. Additionally, LCR 1p36A shows homology to a number of chromosome ends, demonstrating segmental duplication of the 1p telomere-associated repeats. In a similar manner, a second LCR (LCR 1p36C) was identified ~2.2 Mb from the 1p telomere. This LCR is 10.8 kb in size and is found in two copies in an inverted orientation. We also examined the evolutionary conservation of 1p36 LCRs in primate cell lines and reference DNA from human, hamster and mouse. Our results reveal a complex evolution of LCR 1p36A during primate speciation. We propose a model for the evolution of LCRs in 1p36. Of the 95 monosomy 1p36 cases ascertained to date, eight (8.4%) were classified as interstitial deletions. Seven of the interstitial deletion breakpoints and three terminal deletion breakpoints are located within this distal LCR region. Our results suggest a role for genomic architectural features in the generation of terminal deletions of 1p36.

Chromosomal, molecular and morphological analysis of a human embryo with homogeneous trisomy of chromosome 8. *G. Goudefroye^{1,2}, J. Guirchon², C. Ozilou², N. Morichon-Delvallez², A. Munnich¹, M. Vekemans^{1,2}, T. Attié-Bitach^{1,2}, H. Etchevers¹.* 1) INSERM U393, Hôpital Necker Enfants Malades, 149 rue de Sèvres, 75743 Paris Cedex 15, France; 2) Service de Cytogénétique, Hôpital Necker Enfants Malades, 149 rue de Sèvres, 75743 Paris Cedex 15, France.

Our group studies developmental gene expression in normal human embryos obtained from voluntary interruptions of pregnancy, in accordance with ethical guidelines established by French law. A chromosomal anomaly was suspected in one human embryo at Carnegie stage 11 (third week of development) on the basis of abnormal morphology. The embryo had severe malformations of the cardiovascular and central nervous systems. The karyotype of its chorionic villus sample showed a homogeneous trisomy of chromosome 8, whereas FQ-PCR analysis confirmed the meiotic origin of the non-disjunction.

Only a few cases of human trisomy 8 have been reported to date in children or adults. With one recent neonatal exception, the other previous cases reported all showed mosaicism due to mitotic non-disjunction early during zygotic development. Our case demonstrates that homogeneous trisomy 8 is compatible with implantation and early embryogenesis. Nonetheless, by Carnegie stage 11, the heart was bifid, yolk sac vessels were abnormally dilated, the prosencephalon and branchial arches were absent and the somites were small and irregularly shaped. These severe systemic malformations are not compatible with further development, confirming the lethality of trisomy 8 of meiotic origin. In addition, this case justifies the systematic karyotyping of human embryos used for research purposes, as we have observed other trisomies in apparently normal cases; likewise, chromosomal abnormalities may be responsible for other occasional malformations in the embryos we study.

Detection of Turner syndrome with high-throughput quantitative DNA analysis. *J.R. Gruen¹, H. Meng¹, K. Hager², S. Rivkees¹.* 1) Department of Pediatrics, Yale Child Health Research Center, Yale Univ School of Medicine, New Haven, CT; 2) WM Keck Foundation Biotechnology Resource Laboratory, Yale Univ School of Medicine, New Haven, CT.

Turner syndrome (TS) occurs in 1 in 2,000 to 5,000 live female births each year making it one of the most common chromosomal syndromes. Features include short stature, gonadal dysgenesis, and cardiovascular and renal malformations. TS occurs when an X chromosome is completely deleted (45X; 50% of girls with TS), portions of an X chromosome are deleted, or with sex chromosome mosaicism (46XX:45X). Girls with TS may also have occult Y chromosome sequences that are associated with an increased risk for germ cell tumors. Yet despite an estimated 800 new TS cases diagnosed each year, the majority of girls are not detected until after 10 years of age, resulting in delayed treatment and evaluation. Our goal is to develop a high-throughput, sensitive, and economical approach for the early detection of TS during infancy that can be performed as part of routine newborn screening. We assembled a panel of 20 informative single nucleotide polymorphism (SNP) markers distributed through the X chromosome and 10 on the Y chromosome. We then assessed heterozygosity and quantitative DNA pyrosequencing signal in 17 46XX normal females, compared to 6 45X TS subjects, 11 TS mosaics, and 13 other subjects with various sex chromosome aneuploidies. We also tested markers for the detection of Y chromosome material. With just 10 informative SNP markers from the X chromosome we could identify all 45X TS subjects by detecting loss-of-heterozygosity. We also distinguished all the TS mosaics from normal heterozygous samples by comparing the quantitative pyrosequencing signal strengths of paired alleles in the same subject. We also identified Y chromosome material in subjects where Y fragments were previously identified by karyotype and in one subject where karyotype analysis lacked sensitivity. There were no false positive or false negative outcomes in this small trial. These results suggest that inexpensive high-throughput screening, including detection of mosaicism, is possible for TS using quantitative genotyping approaches.

Interphase FISH mapping of chromosomal rearrangements on paraffin-embedded archival patient material. *L. Harewood, J.A. Fantes, D.R. FitzPatrick.* MRC Human Genetics Unit, Edinburgh, United Kingdom.

Disease-associated balanced chromosomal rearrangements (DBCRs) have proved to be extremely important in the mapping of disease loci and positional cloning of disease-causing genes. There is no apparent loss of genetic material in these cases, although small deletions around the breakpoints cannot be excluded. This suggests that the phenotype arises from direct interruption of one or more genes or regulatory elements. The identification and molecular characterisation of breakpoints in individuals with de novo DBCRs can help identify new disease genes or loci. Generally, this type of mapping is done using fluorescent in-situ hybridisation (FISH) on metaphase preparations. However, these are not always available. In this study, we have performed FISH on nuclei dissociated from archive paraffin embedded tissue sections from two cases with distinct phenotypes and de novo, apparently balanced translocations: namely, a t(1;2)(q41;p25.3) with bilateral renal dysplasia and a t(2;12)(p25.1;q23.3) with upper limb peromelia and lower limb phocomelia. By isolating nuclei and performing FISH using BAC probes combined with chromosome specific paints, the breakpoints were mapped to within a few hundred Kb. The genes in the breakpoint regions could then be examined and those that were considered good candidates studied further. This method excludes the need for viable patient material, allowing the study of more cases and hence the identification of further disease genes or loci.

Molecular cytogenetic identification of a cryptic terminal deletion of 15q. *K. Harrison¹, I. Sumargo¹, C. Forster-Gibson^{1,2}*. 1) Dept Pathology & Molecular Medicine, Queen's University, Kingston, ON, Canada; 2) Dept Family Medicine, Queen's University, Kingston, ON, Canada.

We report a 27 year-old male with a cryptic terminal deletion of 15q26.3-qter. He was born at 36 weeks gestation weighing 4 pounds 5 ounces. Miscarriage was threatened at 5 months. As a neonate he had a poor suck. Developmental milestones were globally delayed. As an adult he had short stature; relative macrocephaly; triangular facies; a prominent forehead; a short, narrow nose with a pinched appearance; high arched palate; a central dimple in his chin; brachydactyly; broad hands and feet; hyperextensible fingers and prominent distal interphalangeal joints. As a teen, he had elective mutism and continues to have episodic aggression, destructive behaviour and self-abuse. He has been treated for depression. He had hyperlipidemia but not diabetes mellitus. A skeletal survey and CT scan of the head were normal. There were no identified congenital malformations. Cytogenetic evaluation of lymphocytes identified a 46,XY karyotype (550 bands). Fluorescence in situ hybridization (FISH) screening for a subtelomere rearrangement was requested. Hybridization with subtelomeric probes (ToTelVysion, Vysis) identified a deletion of 15q26.3. A subsequent review of the G-banded metaphases confirmed the deletion to be truly cryptic, not identifiable by retrospective analysis. The karyotype was revised to 46,XY.ish del(15)(q26.3-qter)(D15S936-). Parental chromosome studies have been requested. Cases of distal 15q deletions have been reported to have craniofacial abnormalities, IUGR, developmental delay, and failure to thrive. Ring 15 syndrome involving distal 15q deletions, presents with growth retardation, variable mental retardation, microcephaly, hypertelorism, and triangular facies resembling Russell-Silver syndrome. Our patients clinical features, such as short stature, developmental delay, and triangular facies reminiscent of Russell Silver syndrome, overlapped with the phenotypes described for ring 15 syndrome and cytogenetically visible distal 15q deletions. We believe this is the first description of a submicroscopic deletion of 15q26.3-qter identified by FISH analysis.

Confined placental mosaicism in cases with intrauterine growth retardation of unknown causes. *K. Hiraki, H. Masuzaki, K. Miura, S. Shoko, Y. Fujimoto, T. Ishimaru.* Dept OB/GYN, Nagasaki school of Med, Nagasaki, Nagasaki, Japan.

Objective: Confined placental mosaicism (CPM), which is defined by the presence of abnormal karyotypes only in the placenta while the fetus itself is usually diploid, has been reported to be detected in 1-2% of chorionic villus sampling (CVS) at 10-12 weeks of gestation, and in over 20% of pregnancies with intrauterine growth retardation (IUGR). In this study, the frequency of CPM in cases with IUGR of unknown causes was investigated, and the relation between CPM and IUGR was discussed. **Material and Methods:** To identify CPM, we first screened 50 patients whose infants had IUGR ($-1.5SD$) of unknown causes. Routine screening of other risk factor for IUGR (for example, fetal infections, TORCH syndrome, pre-eclampsia or cord factor) was negative in all cases. Neonatal and placental blood was collected and tissue samples were obtained from at least two sites of placentas at delivery. Standard karyotyping was then carried out. Cellular DNA was extracted from leucocyte of the neonate and their parents, and was genotyped at various dinucleotide repeat polymorphic loci (ABI linkage mapping set MD10). To identify uniparental disomy (UPD), parent-child transmission pattern of polymorphic markers was analyzed. ABI377 autosequencer was used for above genotyping study. **Results:** Nine of 50 cases (18%) examined were diagnosed to have CPM, while UPD was excluded in all cases with CPM. **Conclusion:** CPM was detected in 18% cases with IUGR of unknown causes, suggesting that CPM could be contribute to cause of IUGR though how CPM causes IUGR remains unknown. As UPD was excluded in all cases with CPM, infants IUGR analyzed here may be contributed to other factors, probably to placental dysfunction.

Heterozygotes for the microinversion of the Williams-Beuren syndrome region have an increased risk for affected offspring. *H.H. Hobart¹, R.G. Gregg², C.B. Mervis³, B.F. Robinson⁴, K.W. Kimberley¹, C.M. Rios¹, C.A. Morris⁵.* 1) Molecular Cytogenetics Laboratory, Dept. Pediatrics/Genetics, University of Nevada School of Medicine, Las Vegas, NV; 2) Dept. Biochemistry and Molecular Biology and Center for Genetics and Molecular Medicine, University of Louisville, Louisville, KY; 3) Dept. Psychological and Brain Sciences, University of Louisville, Louisville, KY; 4) Dept. of Psychology, Georgia State University, Atlanta, GA; 5) Dept. Pediatrics/Genetics, University of Nevada School of Medicine, Las Vegas, NV.

Williams-Beuren syndrome (WBS) is a genomic disorder, a contiguous gene microdeletion syndrome, with a prevalence of 1/7500. The typical microdeletion of the WBS chromosome region (WBSCR), the "classic" deletion, includes about 1.6Mb of sequence and 21 genes. The WBSCR is bounded by blocks of similar repeats that likely predispose to deletion due to mismatch pairing. Microinversions of the WBSCR also have been observed. While the parental inverted chromosome has been shown sometimes to be subsequently deleted in WBS individuals, previous sample sizes were not sufficient to determine if parental inversion increased the risk for occurrence of a deletion. The purpose of the present study was to address this question. Samples from 171 2-parent families and 23 1-parent families and their child with classic WBS were studied to determine parental inversion status and parent of origin of the deleted chromosome. Results indicated that: 1) the estimate of population frequency of the WBSCR inversion, based on inversion status in the non-transmitting parent, is 5.06% (95% CI: 1.8%-8.3%). 2) There is no sex difference in inversion frequency. 3) The transmitting parent is equally likely to be male or female. 4) The frequency of the inversion in transmitting parents is 22.5% (95% CI: 16.5%-28.4%). 5) A person heterozygous for the WBSCR inversion has a significantly increased risk for offspring with WBS (odds ratio: 4.45, 95% CI: 2.21-8.96), though few cases of recurrence of WBS have been reported.

Prenatal diagnosis of supernumerary marker chromosomes derived from chromosome 6. *B. Huang^{1,2}, P. Pearle³, J. Philipson³, P. Cotter⁴.* 1) Genzyme Genetics, Orange, CA; 2) Division of Human Genetics, University of California Irvine, Irvine, CA; 3) California Pacific Medical Center, San Francisco, CA; 4) Children's Hospital Oakland, Oakland, CA.

Supernumerary marker chromosomes (SMC) are relatively common in prenatal diagnosis. As the clinical outcomes vary greatly, a better understanding of the karyotype-phenotype correlation for different marker chromosomes will be helpful for predicting the phenotype. We present two cases of prenatally detected small marker chromosomes derived from chromosome 6. In the first case, amniocentesis was performed due to abnormal maternal serum screening indicating an increased risk for neural tube defect. Chromosome analysis showed a 47,XY,+mar[12]/46,XY[10] karyotype. In the second case, the patient was referred for amniocentesis due to advanced maternal age. Chromosome analysis revealed a 47,XX,+mar[6]/46,XX[10] karyotype. The sizes of the marker chromosomes in these two cases appear to be similar. The pregnancies were continued and the babies were born. The presence of the marker chromosomes were confirmed in peripheral blood chromosome analyses. Fluorescence in situ hybridization studies with a chromosome 6 painting probe and a chromosome 6 alpha satellite probe identified the marker to be chromosome 6 in origin in both cases. The clinical manifestations seen in the first baby include small for gestational age, feeding difficulties at birth, hydronephrosis, deviated septum and dysmorphic features, while minimum phenotype was observed in the second baby. The difference in the clinical presentation in these two patients may have resulted from the difference in the actual gene contents of the marker chromosomes and the differential distribution of the mosaicism.

Analysis of a cryptic unbalanced translocation t(17;18)(p13.3;q23) identified by subtelomeric FISH and defined by array-based comparative genomic hybridization [CGH] in a patient with mental retardation. *K.S. Hwang^{1, 2}, P. Pearson³, P.A. Lennon¹, M.L. Cooper¹, J. Wu¹, Z. Ou¹, P. Stankiewicz¹, W.W. Cai¹, S.W. Cheung¹.* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Obstetrics and Gynecology, Tri-Service General Hospital, Taipei, Taiwan; 3) St. Josephs Hospital Laboratory, Phoenix, AZ.

The development of molecular cytogenetics techniques has made it possible to identify microdeletions and cryptic chromosome rearrangements in mentally retarded and dysmorphic individuals who could not be diagnosed by conventional cytogenetics analysis. We report on a 3-year-old girl who presented with mental retardation, growth deficiency, speech delay, and dysmorphic features including hypertelorism, up-slanting palpebral fissures, midfacial hypoplasia, and superiorly pointed ears. The initial G-banding analysis performed by another laboratory revealed a 46,XX,t(3;8)(q26.2;p21.1)mat chromosome pattern. However, the clinical features were suggestive of the 18q-syndrome. Subtelomeric FISH analysis revealed an additional submicroscopic chromosome aberration. A derivative chromosome 18 was identified that resulted from a deletion within chromosome 18 at band q23 and replacement by a third copy of a chromosome 17p region at band p13.3. Subsequent array-based CGH with an extended set of subtelomeric BAC and PAC clones confirmed the identified abnormality and defined more precisely the size of the terminal 18q23 deletion and the 17p13.3 duplication. Molecular cytogenetics characterization of the cryptic rearrangement with these methodologies and follow up studies will be presented. This case further demonstrates the diagnostic usefulness of combining conventional cytogenetics with new molecular chromosome analysis methods for clinical interpretation of subtle chromosome abnormalities.

Molecular analysis of an inv dup(15) demonstrating localization and sequence of the precise breakpoint region and suggestion of the underlying mechanism. *L. Jeng¹, M. Eichenmiller¹, A. Wandstrat², S. Schwartz¹.* 1) Case Western Reserve Univ and Univ Hosp of Cleveland, Cleveland, OH; 2) Univ Texas Southwestern, Dallas, TX.

Inv dup(15) is the most common supernumerary marker chromosome (SMC) and is seen in 50-60% of SMC cases. Molecular analysis has shown maternal origin in all large de novo cases and breakpoints localized most commonly to 15q11-q14, with at least four different breakpoints suggested. Available data suggests involvement of low copy repeats (LCRs) in the PWS/AS region in formation of smaller inv dup(15), but little is known about formation of the largest class of this marker.

In order to further characterize a larger inv dup(15) breakpoint and elucidate the underlying mechanism, we analyzed one inv dup(15) isolated within a somatic cell hybrid using PCR amplification of microsatellites, sequence analysis of single nucleotide polymorphisms, and long-range PCR (LR-PCR) in conjunction with data available on the UCSC Genome Browser on Human. We successfully isolated junction fragments spanning the breakpoint using three PCR primer sets and sequenced over the breakpoint in one of these. Initial studies on a second inv dup(15), with a similar breakpoint cytogenetically, isolated within a somatic cell hybrid did not show the same junction fragments by LR-PCR. This suggests that while the breakpoint may be in the same LCRs, it may not occur at the exact same site.

These studies are of importance as [1] analysis of an inv dup(15) reveals that its breakpoints occur within LCRs localized to 15q13.2 and 15q13.3; [2] the abnormality is not a precise mirror image since the breakpoints are in two different repeats; [3] the two repeats are within 2 Mb of each other and are 99.7% identical, but are oriented in opposite directions; [4] using LR-PCR it is possible to sequence through the breakpoint demonstrating breaks in a Line element and a Sine element within the two LCRs; and [5] this study conclusively demonstrates that LCRs are important in the mechanism of production of the larger inv dup(15), similar to what is seen for the more proximal LCRs important in Prader-Willi and Angelman syndromes.

Chromosome X instability associated with developmental delay? *S. Josefsberg Ben Yehoshua¹, A. Chun-Hui Tsai¹, J.B. LeRoux³, L. McGavran^{2,3}*. 1) Division of Clinical Genetics and Metabolism, The Childrens Hospital/ University of Colorado Health Sciences Center (UCHSC), Denver, CO; 2) Department of Pathology, UCHSC, Denver, CO; 3) Colorado Genetics Laboratory, Denver, CO.

We report a family with abnormalities of the X chromosome. The proposita, a 31 year old G7 P4 SAB2, developmentally appropriate, pregnant woman, had two second trimester miscarriages. Chorionic villus sampling (CVS) of the fetus showed a 46,X,der(X)t(X;Y)(p22.33;q11.22) karyotype. Fluorescence in situ hybridization (FISH) revealed that SRY is absent, as is Y centromere. Both the subtelomeric Xp/Yp sequences and the STS (Steroid Sulfatase) sequences are lost on the derivative X, and the Kallmann gene locus is present. The breakpoint on the X chromosome is therefore between STS and Kallmann gene loci. The same rearrangement was found in the proposita herself and her youngest daughter; who is 5 years old developmentally appropriate short, microcephalic girl. The third child is a developmentally delayed 11 year old boy, with behavioral problems, tall for the family, small penis and limited supination and incomplete pronation of both fore arms. He was found to have Klinefelter syndrome, karyotype 47,XXY. His degree of developmental delay is worse than expected for Klinefelter syndrome children. Her oldest daughter and second son (different fathers) are developmentally delayed and both have a normal karyotype. A 46,X,der(X)t(X;Y) karyotype was also documented in the abortus material from the miscarriage that was submitted for cytogenetic study. We hypothesize that the developmental delay in the family could be due to an abnormality of the maternal of the non-der(X) chromosome. We hypothesize that the proposita and her youngest daughter are developmentally appropriate due to skewed X-inactivation; and her developmentally delayed children are delayed partially due to the inheritance of mutations on the second, non-rearranged maternal X chromosome. X inactivation studies and FISH studies for the SHOX gene are pending and will be presented at the meeting.

Detection of a deletion of the *FMR1* gene during routine screening for Fragile X. *N.B. Kardon*^{1,2}, *B. Levy*^{1,2}, *R. Kornreich*¹, *L. Edelmann*¹. 1) Department of Human Genetics, Mount Sinai School of Medicine, New York, NY; 2) Department of Pediatrics, Mount Sinai School of Medicine, New York, NY.

In our laboratory, Fragile X testing is performed using a combination of PCR to determine CGG repeat size and Southern blot analysis to distinguish wild-type, pre-mutation and full-mutation status. During routine screening for Fragile X, we identified a 39 year old female with complete lack of methylation at the *FMR1* locus, essentially a male Southern blot pattern, and a single allele size of 19 CGG repeats by PCR analysis. After ruling out the possibility of a laboratory error, cytogenetic analysis was requested and a GTG banded karyotype was obtained. The karyotype was determined to be 46,XX,del(X)(q27q28). Therefore, the Fragile X results are explained by deletion of the *FMR1* locus with skewed inactivation of the deleted X chromosome in peripheral blood. Fluorescence in situ hybridization (FISH) using an Xq/Yq TelVysion probe (Vysis) indicated that the deleted X chromosome did not contain the X telomere sequences. Comparative genomic hybridization (CGH) confirmed the deletion of the Xq27 and Xq28 bands, but did not reveal any additional gain of material. The patient has a normal medical history and has given birth to a normal male child and has had at least two first trimester spontaneous abortions. Her family history revealed that she has a normal brother and her mother had two or three miscarriages. Her mother was 42 at menopause. Cytogenetic analysis of GTG banded chromosomes indicated that her mother was carrying the same deleted X chromosome without an apparent additional abnormality, such as a balanced translocation with the X chromosome. We are currently trying to determine whether the deleted X arose through a translocation in a grandparent or whether it represents a true terminal deletion. To our knowledge this is the first report of an X deletion being identified through routine carrier screening for Fragile X, and also represents a new case of stable transmission of a deleted X chromosome from mother to daughter. Presumably, the lack of phenotypic expression from this deletion in both generations is due to skewed X inactivation of the deleted X chromosome.

Maternal paracentric inversion of chromosome 9 and recurrent pregnancy loss. *W.G. Kearns¹, R. Pen¹, D.E. Tourgerman², S.F. Hoegerman³.* 1) Ctr for Preimplant Genet, Rockville, MD; 2) USC Reprod Endo & Infer, Los Angeles, CA; 3) Dept of Biol, College of William & Mary, Williamsburg, VA.

Paracentric inversion carriers are at risk of producing genetically unbalanced gametes. These include a dicentric, an acentric fragment, the normal chromosome or the inverted chromosome. Cells with the dicentric or acentric fragment will eventually become monosomic due to chromosome breakage or loss. Embryos containing cells with a dicentric or acentric fragment of chromosome 9 may not implant or result in fetal loss due to eventual monosomy of chromosome 9. We performed IVF and PGD on a couple due to multiple losses. She is a carrier of a paracentric inversion [46, XX, inv(9)(q21.2q22.3)]. PGD-aneuploidy was performed for chromosomes 9,14,15,22,X & Y. We next performed PGD for telomeric sequences of chromosome 9. Experimental conditions and fluorescence microscopy was performed according to routine protocols. 11 of 13 embryos were aneuploid. 7 of 11 had three chromosome 9 centromeres. Expected paracentric segregation does not predict trisomic cells, therefore 9p and 9q telomere sequences were analyzed to determine the presence of a dicentric chromosome 9. 5 of 7 trisomy 9 blastomeres showed three 9p and one 9q sequence. The other two showed two 9p and two 9q sequences. Each of the remaining (n = 6) diploid chromosome 9 embryos tested showed the correct number of 9 telomeric sequences. Dicentrics whose centromeres are joined by their q arms will have two p-arm and no q-arm telomeric sequences. Cells with a dicentric and a normal chromosome will therefore have a total of 3 p-arm and one q-arm telomeres. This was found in 5 of 7 embryos with three chromosome 9 centromeres. The apparent presence of a dicentric chromosome in a high percentage of embryos suggests the possibility that the recombinational event took place during a mitotic division in the maternal germ line. To our knowledge, this is the first report of a dicentric chromosome in embryos from couples undergoing IVF-PGD and the first suggesting mitotic recombination as a mechanism for the generation of a dicentric chromosome. This dicentric chromosome may play a role in the couples recurrent pregnancy loss.

Maternal cell contamination in cord blood in a newborn baby with ambiguous genitalia: a warning. *A. Khan¹, D. Whelan^{1,2}, J. Xu²*. 1) Pediatrics; 2) Pathology and Molecular Medicine, Hamilton Health Sciences and McMaster University, Canada.

A newborn baby was referred to clinical genetics because of ambiguous genitalia, imperforate anus, bilateral hydronephrosis, club feet, leg contractures and dysmorphic facial features. Analysis of 50 cells from the cultured cord blood showed a mosaicism of 46,XY in 45 cells and 46,XX in 5 cells. While this mosaic karyotype was considered to be consistent with the clinical finding of the ambiguous genitalia, a follow-up confirmation using skin biopsy and peripheral blood was recommended. Of 100 cells from cultured skin tissue, 98 had a 46,XY and 2 had a 45,X. These two 45,X cells were interpreted to be a result of random loss of the Y chromosome from the original normal male cell line. Examination of 100 cells from cultured peripheral blood all had 46,XY. FISH using an SRY probe at Y11.3 showed no evidence of anomalies of this locus in all 10 cells examined. While a cause for the presence of multiple congenital anomalies is yet to be established, we conclude that the female cell line in the cord blood originated from maternal cell contamination (MCC). MCC can go undetected in cases with a female karyotype when cord blood is used for analysis. Our case illustrates that caution should be exercised in interpretation of a finding of mosaicism for male/female cell lines from use of cord blood, especially in cases of ambiguous genitalia. Confirmation using other tissues and supplemental tests such as FISH for SRY locus should be considered for accurate diagnosis and clinical management.

Applications of personalized molecular cytogenetic diagnostics. *J.H.M. Knoll¹, P.K. Rogan^{1,2}*. 1) Children's Mercy Hospital, Schools of Medicine and; 2) Computer Science and Engineering, University of Missouri-Kansas City.

Subtle variations in chromosomal abnormalities are increasingly recognized as having profound impacts on the diagnosis and prognosis of congenital and acquired cytogenetic disorders. Routine distinction of such differences among individuals with the same diagnoses is now feasible with molecular cytogenetic methods as a result of the completion of the euchromatic human genome DNA sequence. We have introduced a new type of reagent for definition of chromosome abnormalities at extremely high resolution for virtually any euchromatic sequence. Single copy (sc) DNA probes, designed by computational analysis of the genome reference sequence, have been developed and validated for >40 inherited and acquired diseases and >80 distinct chromosomal regions, including subtelomeric domains. Probe synthesis involves determining the boundaries of single copy intervals within a specified disease locus, amplifying one or more of these intervals, then purifying and labeling of the amplicon. Sc probes of >2 kb occur on average every 22-31 kb, which is similar to the best resolution obtained by genomic Southern analysis and other methods. scFISH probes can be custom designed and produced rapidly and at a modest cost. In this study, molecular cytogenetic heterogeneity is demonstrated with scFISH in patients having the same clinical diagnosis, although heterogeneity is not evident in these patients using commodity cytogenetic reagents. Furthermore, the repertoire of available commercial reagents is often not optimized for diagnosis of private or infrequent chromosomal rearrangements. This study demonstrates variations in microdeletion size in the Smith-Magenis and Angelman/Prader-Willi syndromes, and precisely delineates chromosome breakpoint intervals in Chronic Myelogenous Leukemia. We have also developed a set of subtelomeric scFISH probes in close proximity to chromosome termini to improve detection of translocation carriers and unbalanced rearrangements. Information obtained with these probes can, in some instances, predict clinical outcomes within subgroups of patients defined by these cytogenomic abnormalities.

Syndromic hearing loss associated with a familial inversion of 7q. *K. Kocher*¹, *C.C. Morton*^{1,2}. 1) Harvard Medical School, Boston, MA; 2) Brigham and Women's Hospital, Boston, MA.

Hearing loss is a common disorder affecting about one per thousand newborns with half of all cases attributed to genetic causes. Hundreds of syndromic forms of hearing loss have been described; however, in many cases the underlying genetic mutations have not yet been identified. Here we report a family with five members spanning three generations who have partial hearing loss and craniofacial defects, including micrognathia and abnormal pinnae. GTG-banding showed that all affected family members carry a balanced paracentric inversion of 7q inv(7)(q21.3q35). FISH experiments were performed to further define the breakpoints on the derivative chromosome 7. Successive FISH experiments, using BACs as probes, were used to identify split BACs that hybridized to both 7q21.3 and 7q35 on the derivative chromosome 7. The FISH analysis mapped the 7q21.3 breakpoint within the BAC RP11-879e11 and the 7q35 breakpoint within the BAC RP11-643a21. Southern blot analysis using PCR products as probes is currently being performed to further refine these breakpoints. The 7q35 breakpoint disrupts the contactin-associated protein-like 2 (*CNTNAP2*) gene between exons 8 and 9, while the 7q21.3 breakpoint lies approximately 70 kb and 85 kb upstream of the *DLX6* and *DLX5* genes, respectively. *DLX5/6* are interesting candidate genes as *DLX5* knock-out mice have inner ear defects along with craniofacial abnormalities (Acampora et al. 1999, Depew et al 1999). Further analysis of *DLX5/6* and *CNTNAP2* is planned in order to identify the gene causing hearing loss in this family.

Clinical phenotype of microdeletions at 3p24 and 5p12 detected by whole genome microarray. *M. Koochek¹, C. Harvard², P. Malenfant³, S. Creighton¹, JJA. Holden^{3,4}, E. Rajcan-Separovic², MES. Lewis¹, and the ASD-CARC; www.autismresearch.ca.* 1) Depts of Medical Genetics; 2) & Pathology, University of British Columbia, Vancouver, Canada; 3) Depts of Physiology; 4) & Psychiatry, Queen's University, Kingston, Canada.

Cri du chat syndrome (CDCs) is a well defined clinical entity with an incidence of 1/15,000-1/50,000. The critical region (CR) for CDCs has been mapped to 5p15, with the hallmark cat-like cry sublocalized to 5p15.3 and the remaining clinical features to 5p15.2. We report findings in a patient with a complex chromosomal rearrangement and a cryptic microdeletion of the CDC-CR detected using a 1Mb genomic array. In addition to 5p-, derived from a de novo paternal translocation between 5p15.2 and 7p13, the proband has a single clone loss at the 3p breakpoint of a de novo paternal inv(3)(p24q24). Deletions were confirmed using microsatellite analysis and FISH. The 5p deletion encompasses ~2.2 Mb mapping to the border between bands 5p15.2 and 5p15.3. The single clone deletion maps to 3p24.3-3p25, for which there is no known phenotype. The clinical features of our proband differ from the characteristic CDC phenotype, which may reflect the effect of both microdeletions and/or further refine the CDC-CR. Typical features of CDC that are present in the proband include moderate intellectual disability, speech delay and dysmorphic features (e.g., broad and high nasal root, hypertelorism and coarse facies). Expected CDC features that are not present are: growth delay, microcephaly, round facies, micrognathia, epicanthal folds, motor delay and the signature high pitched cry. Behavioural traits include ADHD and unmanageable behaviour including, aggression, tantrums, irritability and self destructive behaviour. Several of these behaviours have been previously reported in patients with 5p-. Although most agree on the cat cry critical region there is discrepancy in the location of the region associated with the more severe manifestations of CDCs. The clinical description of this proband and the characterization of his 5p deletion further refine phenotype-genotype correlations in CDCs. Supported by research grants from CIHR and OMHF.

Two unrelated patients with rare interstitial deletion of 9q with mild dysmorphic features. A. Kulharya, S.M. DeLany, D.B. Flannery. Dept Pediatrics, Medical Col Georgia, Augusta, GA.

Interstitial deletions in the long arm of chromosome 9 are rare. The few cases reported in literature do not delineate a characteristic phenotype among patients with overlapping deletions of 9q. We report two unrelated female patients with a *de novo* deletion in 9q. Previous chromosome analysis in both patients was reported as normal elsewhere. In one of these two patients, the deletion was initially identified by whole genome microarray comparative genomic hybridization (array CGH). The first patient was seen in the genetics clinic at 4 months of age because of developmental delay and dysmorphic features. Height and weight were below the 2nd percentile; head circumference was at 15th percentile. No organomegaly was observed; cardiac exam was normal. Chromosome analysis at our lab demonstrated a karyotype of 46,XX,del(9)(q32q34.1). The second patient had been followed for three years by various specialties before being seen in our genetics clinic at three years of age. The pregnancy had been complicated by oligohydramnios and IUGR. MRI showed a slight decrease of white matter in the parietal region. She had been initially diagnosed with abnormal fat distribution and hypothyroidism. She has mild dysmorphic features, developmental delay, seizures and speech delay. A whole genome array CGH using Spectral Chip 2600 (Spectral Genomics) with BAC clones distributed over the entire genome was performed in the second patient. The array-CGH demonstrated the deletion of clones distributed over a distance of 3 Mb (RP11-45A16 and RP11-57K19 to 9q32-q33.3 and q34.11-q34.2 respectively). Retrospective chromosome analysis showed a visible deletion with breakpoints at q32 and q34.1 in chromosome 9. Parental karyotypes in both patients were normal. Of the approximately 20 cases of interstitial 9q deletion reported in literature, only 2 patients have deletions similar to our patient. Based on G-banding pattern, alternative breakpoints for these deletions in 9q are possible. For accurate assessment of a chromosome abnormality, high resolution chromosome analysis and array CGH are sensitive techniques and allow better genotype phenotype correlation. *de novo*.

Characterization of the Region of 4q Associated with Hereditary Benign Intraepithelial Dyskeratosis. *W.S. Lambert¹, J. Clapp², J.E. Hewitt², M. Bembe¹, K. Bastress¹, R. Lemmers³, S.M. van der Maarel³, R. Allingham⁴, J. Vance¹, M.A. Hauser⁵.* 1) Center for Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Institute of Genetics, Queens Medical Center, University of Nottingham, United Kingdom; 3) Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands; 4) Department of Ophthalmology, Duke University Medical Center, Durham, NC; 5) Center for Human Genetics, Department of Ophthalmology, Duke University Medical Center, Durham, NC.

Hereditary benign intraepithelial dyskeratosis (HBID) is an autosomal dominant cell proliferation disorder characterized by opaque epithelial plaques of the conjunctiva and oral mucosa. Redness, irritation, and obstruction of vision secondary to corneal involvement can occur. This disorder has been linked to chromosome 4q and a near-telomeric DNA duplication in this region (Allingham RR, et al. *Am J Hum Genet* 2001). We have evidence that the HBID duplication includes a partial duplication of the D4Z4 repeat located in the sub-telomeric region of 4q. The D4Z4 region has been suggested to play a role in the transcriptional control of 4q genes. We have used RT-PCR and TaqMan gene expression analysis to determine if the partial D4Z4 duplication in HBID results in abnormal expression of 4q genes. Total RNA from a normal conjunctival (CCj) cell line, a HBID ocular lesion (HCj) cell line, normal conjunctival tissue, and HBID ocular lesion tissue, as well as from human-mouse hybrid cell lines containing either the normal chromosome 4 or the chromosome containing the HBID duplication was used in synthesis of first strand cDNA. Polymerase chain reaction (PCR) and real time PCR using TaqMan assays were performed. In addition, genomic real-time PCR was used to determine the HBID duplication boundaries. In general, control cell lines and tissue demonstrated higher expression levels of 4q35 genes than did cell lines and tissue from HBID lesions. Using genomic real-time PCR and other methodologies, we have determined the boundaries of the HBID duplication to within 39 kb. This information will aid us in determining the role of D4Z4 in controlling gene expression in HBID.

Diverse cytogenetic findings in Korean patients with DiGeorge/velocardiofacial syndrome (DG/VCFS)

phenotypes. *J.-Y. Lee¹, S.-J. Yoo^{1, 2}, J.-S. Kim¹, S.-E. Im¹, E.-Y. Choi¹, E.-M. Kwon¹, E.-J. Seo^{1, 2}, H.-W. Yoo^{1, 3}.* 1) Medical Genetics Clinic & Lab, Asan Medical Center, Seoul, Korea; 2) Department of Laboratory Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; 3) Genome Research Center for Birth Defects & Genetic Disorders, Department of Pediatrics, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.

DiGeorge/Velocardiofacial syndrome (DG/VCFS) caused by chromosome 22q11.2 microdeletion shows various clinical features such as conotruncal heart defect (CTHD), abnormal facies, thymic hypoplasia, cleft palate, hypocalcemia due to hypoparathyroidism, developmental delay and mental retardation. However, phenocopies of DG/VCFS are reported in patients with other chromosomal aberrations, dysmorphic syndromes, and malformation syndromes caused by teratogens. From Sep., 1999 to May, 2004, 311 patients with phenotypic or prenatal sonographic features consistent with 22q11.23 microdeletion have been tested by standard cytogenetic and FISH method using TUPLE1 probe from peripheral lymphocytes and prenatal specimen (AF, CB, CVS). Ninety two patients (29.6%) had 22q11.2 deletion. Twenty patients (6.4%) showed no TUPLE1 deletion, but other chromosomal abnormalities. We reviewed the cytogenetic and phenotypic findings of those 20 patients. They revealed diverse karyotypic features including abnormal chromosome 22 (4 patients), partial trisomy 18q (2 patients), del(2q) (2 patients), del(3p), del(4p), dup(4p), del(4q), del(6q), and del(11q). These 20 patients presented with various phenotypic features mimicking DG/VCFS; CT HD (in 13 patients), facial dysmorphism (6), cleft palate (4), developmental delay (5), hypocalcemia (3), renal anomaly (3), hypothyroidism (1), and others. In conclusion, diverse karyotypes were identified in the patients with DG/VCFS phenotypes. These subsets of patients deserve further molecular genetic investigations, which may lead to the discovery of new genetic loci for phenotypic features of DG/VCFS.

Cytogenetic characterisation and phenotype of an unusual supernumerary chromosome(1) marker. *G. Lefort¹, P. Blancher², A.M. Chaze¹, P. Vago³, A. Pinton⁴, F. Pellestor⁵, J. Puechberty², P. Sarda².* 1) Cytogenetics Laboratory, Hopital Arnaud de Villeneuve, Montpellier, France; 2) Department of Medical Genetics, CHU Arnaud de Villeneuve, Montpellier, France; 3) Department of Medical Cytogenetics, CHU Clermont-Ferrand, France; 4) UMR 898, INRA-EMVT, Toulouse, France; 5) CNRS UPR 1142, Montpellier, France.

A 40-year-old gravida 2, para 1 woman was referred for amniocentesis at 16 WG for advanced maternal age. Amniotic fluid chromosome studies diagnosed mosaicism for a " de novo " small supernumerary marker chromosome (SMC) in a male fetus : 47,XY,+mar[13] / 46,XY[8]. M-FISH studies indicated that the SMC contained euchromatic chromosome 1 material, and this was confirmed by positive whole chromosome 1 painting. FISH with probes for centromeric chromosome 1 and subtelomeric chromosome 1p and 1q regions was negative. Microdissection followed by reverse chromosome painting suggested that the marker contained pericentromeric 1p and 1q material. In the light of these results fetal prognosis was discussed with the parents who elected to terminate pregnancy despite normal 20 and 24 WG ultrasound scans. Autopsy of the 25 WG fetus showed slight craniofacial dysmorphism, bilateral camptodactylia and rocker bottom feet, but no organ malformation. Cytogenetic studies on amniotic fluid at termination and on tissues at autopsy (skin, lung, heart, kidney,) confirmed variable mosaicism in all samples. The cytogenetic characteristics and partial trisomy 1 phenotype are discussed .

Detection of subtelomeric rearrangements by FISH. *C. Lozzio, T. Ryan, E. Bamberger, E. Ralston, C. Worthington, W. Carter, M. Huckaba, P. Leopper.* Dept Medical Genetics, Univ Tennessee, Knoxville, TN.

Subtelomeric chromosome rearrangements are one of the causes of mental retardation with or without dysmorphic features and/or congenital anomalies. We studied 673 cases with developmental delay or mental retardation, dysmorphic features and/or congenital anomalies with or without family history of mental retardation and/or miscarriages. We used the Vysis ToTelVysion probe panel. The following cryptic deletions were detected: del 4ptel and 4p16.3 (Wolf-Hirshhorn critical region) in a 2 yrs old female with developmental delay, failure to thrive, dysmorphic features and microcephaly; del 4ptel only in the mother with bipolar disorder and her two children with developmental delay and hyperactivity; del 4qtel in a 4 yrs old female with speech delay and dysmorphic features; del 9qtel in a 9 yrs old female with mental retardation, autistic behavior and dysmorphic features; del 12qtel in a newborn with cleft lip/cleft palate and congenital heart defect; del 13qtel in a 7 yrs old with speech defect, poor motor coordination and dysmorphic features. Two cryptic familial rearrangements were observed: one was a balanced translocation 1ptel;8qtel in a normal 4 yrs old twin of a child with the der(1)t(1ptel;8qtel) unbalanced translocation who had developmental delay, ataxic gait, dysmorphic features and microcephaly; the other familial aberration was a der(4) t(10qtel;4qtel) plus two normal chromosomes 10 causing trisomy 10qtel in a 2 months old with dysmorphic features and in his father with history of learning problems. In this case the 10qtel was translocated to the 4qtel without loss of either telomere. A "de novo" der(13)t(5p;13q) was found in a 4 yrs old with developmental delay, microcephaly and Factor VIII deficiency. Two cases had rearrangements of the sex chromosomes: one was a del Yqtel and dup Yptel in a 4 yrs old with speech delay, dysmorphic features and bilateral radioulnar synostosis; the other case was a del Xqtel replaced by the XpYptel and no signals for STS or KAL at Xp22.3 in a newborn female with polydactyly. Phenotype karyotype correlations and hypothesis on the mechanisms of telomere breakage and rejoining are important aspects of this study.

Disruption of *ROBO2* in a patient with Vesicoureteral Reflux (VUR). W. Lu^{1,8}, R.E. Peters¹, H.L. Ferguson¹, F. Quintero-Rivera^{2,8}, R. Eisenman³, D. Sanlaville⁴, S.A. Feather⁵, A.S. Woolf⁶, M.R. Eccles⁷, D.J. Harris^{3,8}, A.H. Ligon^{1,8}, B.J. Quade^{1,8}, G.A.P. Bruns^{3,8}, J.F. Gusella^{2,8}, C.C. Morton^{1,8}, R.L. Maas^{1,8}. 1) Brigham & Women's Hospital, Boston, MA; 2) Massachusetts General Hospital, Boston, MA; 3) Children's Hospital, Boston, MA; 4) Hôpital Necker-Enfants Malades, Paris, France; 5) St James's University Hospital, Leeds, UK; 6) University College London, UK; 7) University of Otago, Dunedin, New Zealand; 8) Harvard Medical School, Boston, MA.

Vesicoureteral Reflux (VUR) is a common genetic disorder in children with an incidence of ~ 1 in 100. It is characterized by reflux of urine from the bladder into the ureters and kidneys. VUR is found in 30% of young children with a urinary tract infection. These patients may present later in life with proteinuria, hypertension and reflux nephropathy, which collectively account for 15% of cases of end-stage renal disease. VUR results from an anatomic deformity of the ureterovesical junction and has in some cases been suggested to represent an autosomal dominant trait. Despite the high incidence of VUR in the pediatric population, the genetic basis of VUR remains unknown. To identify genes that cause VUR, as part of the Developmental Genome Anatomy Project (DGAP), we studied a patient (DGAP107) with bilateral grade IV VUR and ureterovesical junction defects requiring surgical correction, and a balanced chromosomal translocation 46,X,t(Y;3)(p11;p12). Using fluorescence *in situ* hybridization, we identified a split BAC clone at the 3p12 breakpoint in DGAP107. The breakpoint disrupts intron 2 of the *ROBO2* gene, a human homolog of the *Drosophila roundabout* gene that is involved in axonal pathfinding. By RT-PCR and Northern analysis, *ROBO2* is highly expressed in human embryonic kidney and brain. *Robo2* knockout mice show multiple ureters with supernumerary ureteric buds. Murine *Robo2* has been implicated in the developmental events that restrict ureteric bud outgrowth, and hence kidney induction, to a single site in embryo. DNA sequence analysis of *ROBO2* in familial VUR is underway to test the hypothesis that loss of function variants in human *ROBO2* are causally linked to some cases of VUR.

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Tetrasomy Y syndrome in a child with two idic(Y)(q11.2). *J.T. Mascarello¹, V. Kimonis²*. 1) Genzyme Genetics, Santa Fe, NM; 2) Dept of Genetics, Children's Hospital, Boston, MA.

We present the eighth case of a patient with clinical features of tetrasomy Y syndrome. This patient is the third case attributable to isodicentric chromosomes derived from the Y chromosome and is the first to involve breakpoints in the proximal long arm such that the isochromosome is of a size that could be confused with a structurally normal Y. Like most other patients with tetrasomy Y syndrome, this patient was mosaic, with 25% of cultured lymphocytes containing one isodicentric Y and 75% containing two. At nine years of age, clinical features exhibited by our patient that have also been noted in multiple other patients with tetrasomy Y included: developmental delay, impulsive behavior, prominent alveolar ridges, long philtrum and skeletal abnormalities comprised of radio-ulnar synostosis, asymmetric limb length and fifth finger clinodactyly.

No implication of 8p22-p23.1 duplication in 28 Japanese patients with Kabuki make-up syndrome. *N. Miyake*^{1,2,3}, *N. Harada*⁴, *O. Shimokawa*^{1,4}, *N. Sasonkina*¹, *A. Ookubo*¹, *N. Matsumoto*⁵, *N. Niikawa*^{1,3}. 1) Department of Human Genetics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; 2) Department of Pediatrics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; 3) CREST, Japan Science and Technology Agency, Kawaguchi, Japan; 4) Kyushu Medical Science Nagasaki Laboratory, Nagasaki, Japan; 5) Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan.

Kabuki make-up syndrome (KMS) was characterized by peculiar facial appearance resembling the Kabuki actors make-up, mild to moderate mental retardation, skeletal abnormality, postnatal growth retardation, and dermatoglyphic abnormality. Many affected organs may imply that KMS is a contiguous syndrome, but its cause remains unknown. In 2003, Milunsky and Huang reported that all of 6 KMS patients they examined had an approximate 3.5-Mb duplication at 8p22-p23.1 revealed by comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH) using 4 BAC clones (RP11-112G9, RP11-252K12, RP11-31B7, and RP11-92C1) as probes. They also suggested that a paracentric inversion, detected by RP11-122N11, separated from the duplicated region may contribute to the occurrence of the disease. We analyzed 28 Japanese cases of typical KMS using 15 BAC clones covering the 8p22-8p23.1 region by FISH. However, none of BACs, except for one, showed duplicated signals in any of our cases. Exceptional RP11-122N11 displayed a duplicated pattern of signals in all KMS patients as well as in normal controls, i.e., 15 of 22 KMS cases showed a homozygous duplication and 7 heterozygous, and thus the allele frequency for the duplication is 0.84 in KMS. Similarly, 40 and 12 out of 52 normal controls had a homozygous and a heterozygous duplication, respectively (allele frequency = 0.885). Thus, FISH findings using RP11-122N11 supported that it could detect a duplication polymorphism. In conclusion, our data suggest that the cause of most KMS patients is still unknown, and further studies will absolutely be necessary.

45,X/46,X,psu idic(X)(pter->q26::q26->pter) mosaicism in a patient with premature ovarian failure. *S.K. Murthy, S. Naveed, M.T. Al Ali.* Department of Medical Genetics, Al Wasl Hospital, Dubai, United Arab Emirates.

A 30 years old female with premature ovarian failure (POF) was referred to the cytogenetic lab for chromosome analysis. She appeared to be normal with no stigmata of Turner syndrome. There was no family history of any reproductive abnormalities. Routine G-band chromosome analysis showed an abnormal female karyotype with mosaicism for X-chromosome. Twenty-two of the 45 metaphases analysed had 45,X chromosome complement while the remaining 23 metaphases had 46 chromosomes with one normal X chromosome and one pseudo isodicentric X chromosome involving breakpoint at Xq26. The pseudo isodicentric X was confirmed by C-Banding. X-chromosome mosaicism and/or deletions involving Xq is usually associated with abnormal sexual development and reproductive performance, often associated with POF. Two specific regions on Xq have been defined as POF loci, POF1 at Xq26-q28 and POF2 at Xq13.3-q21. It has been reported that distal deletions involving POF1 results in POF at ages 24-39 years, whereas translocations involving the POF2 locus causes POF at an earlier age of 16-21 years. Detail clinical assessment revealed the presence of possible functional oocytes. The patient opted for IVF and is being followed up. Variant Turner with mosaicism involving pseudo isodicentric (X)(q26) is very rare. Possible mechanism of the origin of the two cell lines and their correlation with POF in our patient will be discussed.

Genetic analysis of male patients referred with infertility. *M.V. Narayana¹, B. Sudheerbabu¹, J.V. Solanki², U. Ratnamala¹, U. Radhakrishna¹.* 1) Molecular Genetics Laboratory, Green Cross Blood Bank & Genetic Research Centre, Ahmedabad; 2) Department of Animal Genetics and Breeding, Veterinary College, Gujarat Agriculture University, Anand-388001, India.

Infertility is a well-known clinical manifestation of various ethical factors and is defined as an inability to conceive after 12 months of unprotected intercourse. The prevalence of infertility couples ranges between 2 and 16%, with 50% of cases due to male infertility. Many cases of male infertility are due to identifiable severe defects in sperm production, which are classified as Azoospermia, Oligospermia, Abnormal motility or morphology and some are idiopathic. Several factors are associated with infertility few of them included infection, immunological factors, anatomic abnormalities, environmental etiologies, endocrinologic dysfunctions and some are influenced by genetic factors. We have analyzed 80 male infertility patients for possible chromosomal anomalies. After the detailed family history and pedigree analysis, routine PHA stimulated peripheral blood cultures were set up and karyotyping was done after GTG banding and other banding techniques were performed as required. A high incidence of 20% (n=16) of chromosomal abnormalities was observed among 80 infertility patients referred (i.e. both structural and numerical). Those included 5% with balanced reciprocal translocations (N=4), 3.75% with sex reversals (n=3) and 12.5% with gonosomal aneuploidies/mosaicisms (n=9). These data suggests that patients with history of infertility should be investigated cytogenetically. DNA microsatellite and FISH analysis are in progress in few patients found with rare chromosomal translocations and also Y chromosome microdeletions analysis. u_c_rao@hotmail.com.

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Molecular cytogenetic characterization of a familial der(1)del(1)(p36.33)dup(1)(p36.22p36.33) associated with a variable phenotype. *J.K. Northup^{1,2}, V.S. Tonk^{3,4}, G.N. Wilson⁵, S. Yatsenko⁶, P. Stankiewicz⁶, J.R. Lupski^{6,7,8}, G.V.N. Velagaleti^{1,2}.* 1) Depts. Pathology and; 2) Pediatrics, University of Texas Medical Branch, Galveston, TX; 3) Depts. of Pediatrics and; 4) Pathology, Texas Tech University, Lubbock, TX; 5) Cook Children's Hospital, Fort Worth, TX; 6) Depts. of Molecular & Human Genetics and; 7) Pediatrics, Baylor College of Medicine, Houston, TX; 8) Texas Children's Hospital, Houston, TX.

Rearrangements involving chromosome 1p36 are very frequent, with deletions occurring at a frequency of ~1 in 5000 live births. Recently, using G-banding and whole chromosome painting, we identified a familial duplication dup(1)(p36.3) in a family associated with variable mental disability, attention deficit-hyperactivity disorder, and a distinctive facial appearance (Tonk et al., 2003). Subsequent subtelomere FISH analysis showed a concomitant terminal deletion in three affected family members. Several BAC and PAC probes mapping to 1p36.22-p36.33 revealed duplication of this region both on metaphase chromosomes and in interphase nuclei. Probes from 1p36.13-p36.21 region showed a normal hybridization pattern. We estimated the size of the deletion to ~550 Kb, and the duplication to ~10 Mb. Interestingly, chromosome 1p36.22 is known to harbor an intriguing gene cluster of locally repeated sequences. Unequal crossing over within these repeats was suggested to be the mechanism for deletions and duplications involving this region. However, recent studies of the del (1)(p36) cases have shown that the breakpoints vary widely and do not cluster. It has been suggested that breakage-fusion-bridge cycles might explain the origin of constitutional terminal deletions associated with interrupted inverted duplications (Ballif et al., 2004). Electronic analysis of DNA sequence in 1p36.33 revealed that this region contains low copy repeats. Further studies are in progress to define the breakpoint junctions and to elucidate the mechanism of this chromosome rearrangement.

A newborn with severe intrauterine growth retardation and dysmorphic features and multiple numerical and structural chromosomal anomalies: a chromosome instability syndrome? *M.J.M. Nowaczyk^{1,2}, J. Xu¹*. 1) Department of Pathology & Pediatrics, McMaster University, Hamilton, Ont., Canada; 2) Department of Pediatrics, McMaster University, Hamilton, Ont. Canada.

A newborn baby boy had multiple dysmorphic features, severe IUGR (weight at 50 centile for 26 weeks gestation 40 weeks), hepatomegaly and thrombocytopenia and anemia requiring transfusions. The parents were 1st cousins once removed and both had a normal karyotype. Routine cytogenetics of the child's blood taken at age of 2 days showed multiple numerical and structural anomalies in all 20 cells examined. At least 9 cells had X and Y chromosomes and 1 cell had 45,X. Chromosome number varied from 43[1], 44[1], 45[5], 46[4], 47[2], 48[2], 69[1] to tetraploidy 92[4]. Structural anomalies included markers in 10 cells, dicentrics in 5 cells, and rings in 2 cells. Of 21 cells examined in a repeat blood taken at age of 13 days, 2 had 46,XY and 19 had similar multiple numerical and structural aberrations. Chromosome number varied: 44[2], 45[5], 46[3], 47[3], 48[6]. Five cells had 1 marker, 6 had 2 markers, and 2 had 3 markers. The markers looked different from cell to cell. Six cells had distal deletions and/or additions, each with del(1)(q11) and del(6)(q11); add(4)(q35) and del(7)(q11.1); del(2)(q22); del(10q); del(12)(q21); del(18)(p11.2). Of 20 cells examined from skin tissue, 15 had 46,XY. The remaining 5 cells each had a different aberration: 45,XY,-20; 45,XY,-17; 43,Y,-X,-8,-11; 45,XY,t(14;15)(q10;q10); 89,XYY,-14,-15,-18. While mosaicism cannot be ruled out, these 5 abnormal cells are likely a result of technical artefact. Our routine analyses so far showed no cytogenetic hallmarks of Roberts syndrome (e.g. puffing at centromere or premature centromere separation (PCS)), ataxia-telangiectasia (increased aberrations involving chromosomes 7, 14 and X), Fanconi anemia (increased chromatid breakages and quadri- or tri-radial figures) or mosaic variegated aneuploidy syndrome (+8, +18, +/-PCS). While this case awaits further cytogenetics and DNA investigations, we welcome any comments and suggestions.

Low-copy repeats and acrocentric heterochromatin mediate a familial chromosome translocation t(14;17)(p13;p12). Z. Ou¹, S.P. Sparagana², M. Jarmuz³, B. Nowakowska^{1,4}, P. Furman¹, L.G. Shaffer^{3,5}, P. Stankiewicz¹, J.R. Lupski^{1,6}, S.W. Cheung¹. 1) Dept. of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Texas Scottish Rite Hospital for Children, Dallas, TX; 3) Health Research and Education Center, Washington State University, Spokane, WA; 4) Dept. of Medical Genetics, Institute of Mother and Child, Warsaw, Poland; 5) Sacred Heart Medical Center, Spokane, WA; 6) Dept. of Pediatrics, Baylor College of Medicine, Houston, TX.

Recently, several molecular studies of chromosome translocation breakpoints revealed nonhomologous recombination as a prevailing mechanism for the formation of nonrecurrent constitutional translocations. However, more cases have to be studied to elucidate the role of the genome architecture in the origin of nonrecurrent chromosome rearrangements. We report the clinical and molecular cytogenetic data of a familial translocation t(14;17)(p13;p12). A 6-year-old girl and her 5-year-old brother were diagnosed with mental retardation, speech delay, short stature, Charcot-Marie-Tooth disease type 1 (CMT1A), large eyes with splotchy melanization of the sclera, long straight eyelashes, down-slanting palpebral fissures, protruding ears, micrognathia, and short neck. G-banding chromosome analysis showed a der(14)t(14;17)(p13;p12) in both siblings, inherited from their father, a carrier of the balanced translocation. Chromosome microarray analysis revealed that the *PMP22* gene was duplicated and mapped the chromosome 17 breakpoint between the Smith-Magenis syndrome and CMT1A chromosome regions, within a breakage prone, ~383 kb low-copy repeat (LCR17pA). Subsequent FISH studies narrowed the 17p breakpoint to BAC clone CTD-3157E16. Interestingly, this clone was previously identified at the breakpoint of the evolutionary chromosome translocation t(4;19) in gorilla. The chromosome 14p breakpoint was mapped distal to the ribosomal DNA, outside the region commonly rearranged in Robertsonian translocations. Our data support previous observations that higher-order genomic architectural features such as LCRs and repetitive DNA sequences, play a role in the origin of nonrecurrent chromosome translocations.

Unexpected Fertility In Men with Robertsonian Translocation (22q;22q) and a New Paternal Uniparental Disomy 22. *K. Ouldim, A. Sbiti, H. Natiq, A. Arazam, A. Laamari, F. El-Kerch, A. Sefiani.* Department of medical genetics, INH, Rabat, Rabat, Morocco.

Uniparental disomy (UPD) describes the inheritance of a pair of chromosomes from only one parent and may comprise the whole chromosome or confined to only a small segment. Several conditions have been resulted from UPD. We report in this study an unexpected fertility in a couple with the repeated abortions. They had a boy with a normal phenotype. The cytogenetic analysis of the father and his son revealed the presence of a robertsonian translocation (22q;22q). The mothers karyotype was normal. A molecular study with chromosome 22 microsatellite markers and the paternity test confirmed the inheritance of this paternal translocation with the lack of the maternal 22 chromosome. A few cases of UPD22 are reported : one case of the paternal UPD 22 and three cases of the maternal UPD 22. All have been associated with infertility. Our finding showed that the inherited paternal UPD 22 has no effect on his son phenotype and represented the second case reported in the literature of the total paternal UPD 22 with the normal phenotype. The mechanisms which probably leading to UPD 22 in our case was discussed.

Validation of the Chromosome Microarray analysis (CMA) for detection of the 1.5Mb, 3Mb and 6Mb microdeletions in DiGeorge/Velocardiofacial syndrome. A. Patel¹, M.L. Cooper¹, J. Li¹, Z. Ou¹, W. Yu¹, X. Lu², J. Kim², C. Shaw¹, C. Chinault¹, S.W. Cheung¹. 1) Dept Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Spectral Genomics, Houston, TX.

The 22q11.2 deletion syndrome which includes DiGeorge and velocardiofacial syndromes(DGS/VCFS) is one of the most common microdeletion syndrome with an estimate incidence of 1:4000. There are several sets of low copy repeat (LCRs) genomic elements within the 22q11.2 region which undergo non-allelic homologous recombination during meiosis leading to this frequent microdeletion syndrome. Approximately 87% of the DG/VCFS patients have an identical deletion of 3Mb involving the most proximal and distal LCR. A smaller subset of patients (10%) has a smaller 1.5Mb deletion and even rarer are the atypical deletions or translocations. We have designed a chromosomal microarray analysis (CMA) chip to detect majority of the microdeletion syndromes including the DG/VCFS syndrome. Eight non-overlapping clones spanning the DG/VCFS region were included in the CMA CHIP to detect deletions spanning from 1.5Mb to 6Mb. In a preliminary validation study, five DG/VCFS patients that were known have a deletion of 22q11.2 by FISH analyses using the TUPLE1 probe were studied by our chromosome microarray. The Chromosomal Microarray analysis was 100% concordance with the FISH data and furthermore it allowed the exact delineation of the deletion sizes in these patients. Three patients had the common 3Mb deletion, 1 patient had the smaller 1.5Mb deletion and one patient had a 6Mb deletion. The sizes of the deletions were confirmed by FISH analysis. Thus, the CMA offers a rapid and a high throughput method for accurately detecting the various sized 22q11.2 deletions. DG/VCFS has a wide phenotypic spectrum however, it is thought there is much less heterogeneity at the genetic level. Routine CMA analysis for all DG/VCFS patients will not only allow for an accurate identification of the size of the deletion but will also quickly identify patients with smaller and atypical deletions for a more exact genotype/phenotype correlation and determination of risks associated with the 22q11.2 deletion syndrome.

Molecular cytogenetic and CGH-array studies in patients with autistic disorder. *S. Patil¹, E. Westin¹, R. Frantz², E. Ashley³, J. Piven³, V. Sheffield¹, T. Wassink².* 1) Department of Pediatrics, Div Medical Genetics, University of Iowa, Iowa City, IA; 2) Department of Psychiatry, University of Iowa, Iowa City, IA; 3) Department of Psychiatry, University of North Carolina, Chapel Hill, NC.

Autism is a neurodevelopmental disorder characterized by severe ritualistic-repetitive behaviors, impaired social interaction and impaired communication/language. Based on published cytogenetic studies, it is known that about 5-8% of cases of autism are associated with chromosome abnormalities. As part of an ongoing project on the molecular genetics of autism, we have recruited 76 patients with autistic disorder at the Autism Research Center in North Carolina. So far in these patients, we have performed routine chromosome analysis, as well as FISH analysis, using specific probes for chromosome 15q11.2q13 probes (DS15S11, SNRPN, D15S10, GABRB3), chromosome 22q11.2 probes (N-25 and TUPLE1 in 20 cases), and the subtelomere FISH panel (VYSIS). In addition, CGH-array studies (GenoSensor) have been carried out on 18 of these individuals. Five patients (6.6%) had chromosomal abnormalities [XXY, XY, inv dup (15q) supernumerary chromosome, del(2)(q37.1q37.3) and Y chromosome with chromosome 15 satellites on Yq]. None of the cases showed N-25 or TUPLE1 deletion. The subtelomere FISH panel confirmed 4 of these abnormalities. However, no new rearrangements were detected. The CGH array confirmed all five abnormalities described above, plus four (22%) additional deletions or duplication of certain markers. These include: two patients with deletion of marker D2S447 on 2qtel, deletion of PRKCZ at 1p36.33 in one patient, and duplication of D14S308 at 14qtel in one patient. Details of our experience with the CGH-array will be presented. Other molecular studies are being carried out to confirm these findings. Because of the limitations of detecting subtle abnormalities with routine cytogenetics, application of CGH-array methodology would yield a higher rate of detection of deletions/duplications, and importantly aid in the identification of novel genomic regions and genes involved in autism.

Multiple anomalies among siblings due to unbalanced chromosome 2;17 translocations. *J. Pfothenhauer¹, A. Yenamandra², J.A. Phillips, III¹, M.P. Cohen¹, M.L. Summar¹, V.L. Hannig¹, G.E. Tiller¹.* 1) Div Med Genetics, Vanderbilt Univ Sch Med, Nashville, TN; 2) Genetics Associates, Nashville, TN.

We present 3 sibs with multiple congenital anomalies secondary to a maternal (2;17)(q37;q25) balanced translocation. The couple's first pregnancy resulted in a healthy daughter. The second pregnancy produced a female with VSD, ASD, hypoplastic left heart and aortic arch with coarctation. Karyotype revealed 46,XX, der(2)t(2;17)(q37;q25), with partial monosomy 2q and partial trisomy 17q. Dysmorphic features included brachycephaly, prominent brow, flat facies, upslanting short palpebral fissures, mild synophrys, depressed nasal bridge, long philtrum, thin downturned lips, prominent frenulum, short neck, redundant nuchal skin, and mild 2-3 toe syndactyly. The baby underwent NG tube placement, 2-stage Norwood repair, angioplasty, and a bilateral Glenn shunt. At 5 months of age she contracted RSV bronchiolitis and died of respiratory failure. The father's blood karyotype was normal 46,XY. The mother's blood karyotype revealed the balanced translocation (cited above) in all cells studied. A third pregnancy was miscarried in the first trimester, but no products of conception were retrieved. The fourth pregnancy was found by amniocentesis to have 46,XX,der(2) t(2;17)(q37;q25)mat, the same unbalanced translocation previously found. At delivery, physical exam revealed acrocyanosis, low forehead and low hairline, extremely small fontanelles, hypertelorism, epicanthal folds, upturned nose with depressed nasal bridge, lowset posteriorly rotated ears, micrognathia, and multiple VSDs. By 2 years of age the child exhibited hypotonia, developmental delay, feeding problems, an umbilical hernia, and planovalgus deformities of her feet. The fifth pregnancy culminated in a healthy appearing female infant. Echocardiogram was normal. Physical exam was unremarkable except for small fontanelles and upturned nares. A karyotype revealed an unbalanced translocation 46,XX, der(17) t(2;17)(q37;q25)mat, with partial trisomy 2q and partial monosomy 17q. This family presents the interesting phenomena of 2 reciprocal products of adjacent segregation among 3 independent meiotic events.

Mosaicism involving two structural abnormalities of chromosome 18 in a patient with a mild phenotype. *M. Poscente, D. Turchetti, S. Cugusi, C. Graziano, G. Romeo, M. Seri.* Lab. Medical Genetics, University of Bologna Medical School, Italy.

A pregnant woman was referred for genetic counselling because of an abnormal fetal karyotype at amniocentesis performed for advanced maternal age. The fetal karyotype was 46,XX,del(18)(p11.22pter) and the deletion was detected to be maternal in origin. The pregnancy was terminated at 23 weeks. The clinical evaluation of consultant revealed some typical findings of 18p- syndrome (palpebral ptosis, midface hypoplasia, ophthalmological and dental abnormalities). Moreover, she presented scoliosis and asymptomatic mitral valve prolapse but no evidence of growth and/or mental retardation. Because of milder phenotype compared to described 18p- syndrome, an in-depth karyotype analysis was performed: a 100 peripheral blood metaphases analysis revealed a mosaic karyotype with three cell lines, all with a chromosome 18 structural rearrangement. The final karyotype was defined as: 46,XX,del(18)(p11.22pter)[79]/46,XX,idic(18q)(qterp11.22::p11.22qter)[20]/47,XX,idic(18q)(qterp11.22::p11.22qter)x2[1]. FISH analysis with specific BAC probes localized the breakpoint region in 18p11.22 and confirmed the deletion comprising the 18p subtelomeric region. The patient, therefore, has a homogeneous del(18)(p11.22pter) and a mosaic dup(18)(qterp11.2) because of the isodicentric structure in 20% of cells. Structural chromosome rearrangements are rarely seen in mosaic form with the exception of mitotically unstable chromosomes such as dicentric. The formation of the fused chromosome can be explained by postzygotic exchange of sister chromatids on the short arm of chromosome 18, followed by breakage and U-shape reunion of sister chromatids; the deletion of the short arm is a possible initiating mechanism with dicentric 18 chromosome, as described in studies on i(X). The few abnormal features seen in our patient may represent a mild expression of the 18p- phenotype inhibiting the effects of the mosaic partial trisomy 18.

Genetic analysis of infertile males from Bangalore, India. *M.G. Prabhakara¹, M. Babu¹, M. Ali¹, V. Bajaj¹, G.B. Manjunath¹, S. Khare¹, K.M. Prasannakumar², A. Kumar¹.* 1) MRDG, Indian Institute of Science, Bangalore, Karnataka, India; 2) Department of Endocrinology and Metabolism, MS Ramaiah Medical College, Bangalore, India.

Approximately 13-18% couples of child bearing age are infertile. Male infertility accounts for approximately half of it. Male infertility is known to have a genetic basis in many cases. Several studies have been performed on the cytogenetic and molecular basis of male infertility in patients from the western populations. Recently, four groups from India have independently reported molecular basis of male infertility in cases from Kolkata, Delhi, Hyderabad and Varanasi. However, there is no report on the genetic basis of male infertility in patients from Bangalore. To this end, we have begun to ascertain cases with male infertility from Bangalore. We have ascertained so far a total of 53 cases with male infertility from Bangalore. These cases were either azoospermic or oligospermic. The G-banding chromosome analysis showed a normal male karyotype in all except one case. The karyotype of one infertile patient was 47,XYY. PCR-based microdeletion analysis of the Y-chromosome using a battery of 29 primer sets from AZF regions (viz. sY746, sY740, sY86, sY2320, sY741, sY84, DFFRY, sY742, sY615 and sY743 from AZFa region; sY98, sY100, sY110, sY80, sY113, sY118, sY124, sY127, sY1211, sY134 and sY143 from AZFb region; sY153, sY148, sY156, sY255, sY254 and sY158 from AZFc region; and, SRY from the p-arm and sY160 from the heterochromatic region) showed deletions in four patients (4/53; 7.5%) in the AZFc region only. Interestingly, patients from other regions of India have shown deletions in all three AZF regions. This suggests a regional variation in the deletions of AZF regions in Indian male infertile patients. The sizes of the deletions were variable in these patients. Deleted segment in only one patient extended to the heterochromatic region of the Y-chromosome. (The financial assistance from the ICMR, New Delhi is gratefully acknowledged).

FISH analysis of meiotic segregation in the sperm of a complex chromosome rearrangement carrier. J.

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Complex chromosome rearrangements (CCRs) are rare events involving at least three breakpoints located on at least two chromosomes. They are usually diagnosed during the investigation of infertility or sometimes at prenatal diagnosis. The advent of molecular cytogenetic techniques has led to a better characterization of RCCs and genetic counseling has benefitted by this advance. We report a CCR initially detected by classical cytogenetic techniques and subsequently analyzed by FISH techniques. **CASE REPORT** : Chromosome studies were done following recurrent spontaneous abortions. Blood lymphocyte karyotype in the 49-year-old man showed an apparently balanced translocation with three breakpoints on chromosomes 5, 13 and 14 : t(5;13;14)(q23;q21;q31). In order to evaluate the reproductive possibility of this patient we performed FISH analysis of the meiotic segregation in sperm using region-specific probes overlapping the translocation breakpoints. A preliminary study analysed 218 spermatazoa. The frequency of alternate and unbalanced forms (adjacent 1, 4 : 2 and 5 : 1) were respectively 28% and 72%. Because of the high percentage of unbalanced forms, IVF with PGD were not undertaken. The information obtained from meiotic sperm studies is useful for genetic counseling and is an aid to sterile male translocation carriers when deciding which couples should benefit from assisted reproductive technologies.

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Ring chromosome 9 [r(9)(p24q34)]: a report of two cases. *S.M. Purandare, J. Lee, S. Hased, M.I. Steele, P.R. Blackett, J.J. Mulvihill, S. Li.* Department of Pediatrics, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

We report clinical and molecular cytogenetic studies in two patients with ring chromosome 9. Cytogenetics and fluorescent in situ hybridization (FISH) analyses using the p16 gene probe on 9p21, the ABL gene on 9q34, chromosome 9 alpha satellite-centromeric probes, and TelVision 9p and 9q probes that detect subtelomere-specific sequences on chromosome 9p and 9q revealed 46,XX,r(9)(p24q34).ish r(9)(305J7-T7-,p16+,ABL+, D9S325-) and 46XY,r(9)(p24q34).ish r(9)(305J7-T7-,p16+,ABL+, D9S325-). Based on FISH analysis at least 115 kb was deleted on terminal 9p, and at least 95 kb from terminal 9q. In comparison with other reports of r(9), deletion 9p, and deletion 9q, both patients had clinical characteristics of ring 9 and additional features of deletion 9q or deletion 9p syndrome. The variability between the two cases with r(9) despite similar breakpoints identified by GTG-banding and FISH may be explained by submicroscopic differences between deletion breakpoints, ring instability, interaction of other genes on the phenotype, and variation in fetal environmental conditions.

Clinical and molecular cytogenetic findings resulting from an unusual recombinant chromosome 16 derived from a paternal ins(16). *R. Ray¹, K. Brierley¹, C. Asquith-Smith¹, P. Chakraborty^{1,2}, J. McGowan-Jordan^{1,2}.* 1) Genetics, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada; 2) University of Ottawa, Ottawa, Ontario, Canada.

We report the clinical and molecular cytogenetic findings in a 6-year-old girl referred for a genetic evaluation. Clinically, she has a history of congenital hydrocephalus, moderate developmental delay, hypotonia and autistic features.. Chromosomal analysis on peripheral lymphocytes revealed an unbalanced female karyotype which was initially assigned as: 46,XX,?rec(16)dup(16q). To characterize the rearranged chromosome 16, fluorescence in situ hybridization (FISH) analysis with a series of probes was performed. Using whole chromosome 16 paint showed that the entire abnormal chromosome was comprised of chromosome 16 material. Analysis of additional probes demonstrated that: (1) the CFBF locus (16q22) localized to the long arm (2) the MYH11 (16p13.1) was found to hybridize distal to the CFBF locus in the long arm and (3) the long arm subtelomeric probe, Z96319 was present terminally on both the long arm and short arm of the abnormal chromosome 16. The results indicate that a portion of the short arm from band 16p11.2 to band 16p13.1 was inserted into the long arm of the same chromosome at band q22 resulting in trisomy for the terminal region of the long arm of chromosome 16 (16q22 to 16qter) and monosomy for the terminal region of the shot arm (16p13.1 to 16pter). Analysis of parental peripheral lymphocytes identified the father to carry an ins(16)(q22p11.2p13.1). By FISH, the abnormal 16 chromosome painted completely with the whole chromosome 16 painting probe. Analysis of additional probes, CFBF, MYH11 and Z96319 confirmed this interpretation. Taken together, there appears to be no discernable loss of chromosomal material on the paternal ins(16). Carriers of such chromosomal rearrangements are at increased risk of transmitting an unbalanced chromosome to their offspring. Therefore, his daughter is likely the result of a meiotic event involving the abnormal paternal chromosome 16. Her final karyotype was: 46,XX,rec(16)dup(16q)ins(16)(q22p11.2p13.1).

Prenatally Detected Extra Structurally Abnormal Chromosome: The Dilemma of Genetic Counseling. O.

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We describe a series of 3 cases with prenatally detected Extra Structurally Abnormal Chromosome (ESAC). Cases 1 and 2 were unrelated singletons, case 3 was one of monozygotic twins and all were spontaneously conceived. In all cases amniocentesis was performed due to advanced maternal age and the ESAC was a co-incidental finding. Fluorescent In Situ Hybridization (FISH) analyses demonstrated that all ESACs were bisatellited. Cases 1 and 2 were inv dup (15), and in case 3 the ESAC was derived from either chromosome 14 or 22. Cases 1 and 3 were de novo while case 2 was maternally transmitted. Mosaicism was detected in case 3 only in the range of 80%. FISH studies to the Prader Willi and Cat Eye regions in the inv dup (15) and the 14/22 ESACs respectively, did not show any hybridization signal. In all cases uniparental disomy was excluded. Following an extensive genetic counseling the pregnancy of case 1 was terminated while the other 2 were carried to term. The decision making process particularly of monozygotic twins with hetero karyotypes is presented.

Mental retardation, hyperphagia and obesity due to inherited 22q13.33 deletion. *J. Roberson¹, D. Van Dyke², A. Wiktor².* 1) Dept Medical Genetics, Henry Ford Hosp, Detroit, MI; 2) Mayo Clinic, Rochester, MN.

CJ is a 22 year old female who had been followed since infancy for moderate to severe mental retardation, excessive food-seeking behavior, obesity, aggressive behavior and drooling. She was born after a full-term uncomplicated pregnancy to parents who were moderately mentally impaired. She was adopted by a maternal relative. Birth weight was 7 lb. 12 oz. At age 2 her overall functioning on the Bayley Scales of Infant Development was 10 1/2-11 1/2 months. At age 22 years she is non-verbal, she indicates her wants by pointing or getting the object she wants. Growth was at the 50th percentile for height and weight through age 5 years. Stature remains at the 50th percentile but her weight was 95th percentile by age 8 years. She weighs 231 lb. at age 22 years (markedly above the 98th percentile). Head circumference was above the 98th percentile by age 10 years. She does not have dysmorphic features. She displays aggressive behavior, touch-avoidance, sleep difficulties, excessive food-seeking activity such as stealing food, eating from the garbage and tantrums if she is not given the food she wants. Chromosome analysis, Smith-Magenis FISH testing, Fragile X and Prader-Willi/Angelman syndrome DNA testing and thyroid function studies were normal.

CJ's mother also had negative chromosome analysis and Fragile X testing. She is a 43 year old who also has moderate learning delays, very limited language skills, obesity, and insulin dependent diabetes. No other maternal relatives have mental retardation.

Subtelomeric FISH probes identified a 22q13.33 deletion in mother and daughter. The ARSA probe was not deleted. The previously described phenotype in distal 22q deletion includes generalized hypotonia, severe delay of expressive language, dolicocephaly, abnormal ears, ptosis, dysplastic toenails, and relatively large hands. This patient and her mother demonstrate the previously reported severe language delays; however, their obesity, excessive food-seeking behavior and lack of dysmorphic features differ from the previously documented cases.

Monosomy 1p36/trisomy 5q35.1 in a baby girl with seizures, hypotonia and developmental delay. *G.M. Ronen¹, J. Xu²*. 1) Pediatrics; 2) Pathology and Molecular Medicine and Lab Medicine, Hamilton Health Sciences and McMaster University, Canada.

We describe the first report of monosomy 1p36/trisomy 5q35.1 in a girl who presented initially at 3 months with complex partial and secondary generalized seizures, progressive microcephaly (-4SD), hypotonia, failure to thrive and global developmental delay (DD). Dysmorphic features included low forehead, large cheeks, closely spaced eyes, mildly slanted palpebral fissures, small ears, slight saddle nose, thin upper lip, smooth philtrum, high arched palate, retrognathia and short neck. EEG and cranial ultrasound were normal. Cytogenetics of the blood sample showed that the patient had a de novo unbalanced female karyotype of 46,XX, der(1)t(1;5)(p36;q35.1) with monosomy for 1p36 and trisomy for 5q35.1. This interpretation is based on analysis of G-banding, spectral karyotyping using all 24 whole chromosome paints (wcp) and FISH using wcp5, 1p telomere probe (TelVysion 1p, Vysis) at 1p36, 5p probe D5S23/D5S721 (control probe of LSI probe EGR1) at 5p15, and 5q telomere probe (TelVysion 5q, Vysis) at 5q35. FISH using SNRPN found no evidence of anomalies of Prader-Willi syndrome critical region at 15q11-q13. Monosomy 1p36 is a new syndrome with well recognized features including growth and mental retardation; seizures; visual problems; large anterior fontanelle; asymmetric, low-set, dysplastic ears; deep-set eyes; depressed nasal bridge; pointed chin and fifth finger clinodactyly (Slavotinek et al., *J Med Genet* 1999, 36:657-663). Trisomy 5q35.1 is relatively rare but has been reported often in combination with other chromosome aberrations. While the clinical picture of the trisomy 5q is still emerging, it seems to share many findings with the monosomy 1p, such as DD, seizures, some dysmorphic features (Groen et al., *Am J Med Genet* 1998, 80:448-453). Future case reports would further help establish the karyotype/phenotype correlation and the clinical outcome of such patients.

The contribution of human chromosome 14p sequences to the breakpoint region of 14q21q Robertsonian translocations determined by single nucleotide polymorphism (SNP) analysis. A.L. Rosa^{1,2}, M. Jarmuz¹, K.A. Bailey^{1,2}, C.D. Glotzbach^{1,2}, L.G. Shaffer^{1,2}. 1) Health Research and Education Center, Washington State University, Spokane, WA; 2) Sacred Heart Medical Center, Spokane, WA.

Robertsonian translocations (ROB) are the most common chromosomal rearrangement in humans with 1:1000 carriers in the general population. The contribution of chromosome 14 sequences to ROB breakpoints is of particular interest because rob(13q14q) and rob(14q21q) constitute about 86% of the total ROB. We have performed high-density PCR-based marker walking over a ~330 kb DNA sequence containing extensive segments of homology between the p-arms of the various acrocentric chromosomes. The region is suspected to contain the physical site for the occurrence of most of the ROB breakpoints. The analyzed sequences are rich in highly repetitive DNA elements. A surprisingly dense concentration of single nucleotide polymorphisms (SNPs) in paralogous copies of STSs was recognized in a subregion of ~12 kb. Using fully sequenced BACs, multiple linked SNPs were defined in short DNA fragments (100-300 bp) distributed over a large interval of ~300 kb. Patches of this region are contained at the p-arm of the various acrocentric chromosomes. The precise chromosomal origin of the analysed BACs (13p, 14p, 15p, 21p or 22p) was unknown. Extensive analyses of the defined SNPs were used to define arbitrary haplotype phases for the various BACs. The chromosomal origin of each BAC was assigned by determining equivalent individual SNP haplotypes on human acrocentric chromosomes carried by monochromosomal hybrid cells. Results of BAC chromosome assignments were confirmed by FISH analyses. The availability of SNP haplotypes allowed us to study partial 14p and 21p chromosome contributions to the breakpoint region in 14q21q ROB. SNP analyses of hybrid cells carrying single *de novo* ROB chromosomes indicate that a large part of the breakpoint region in 14q21q ROB is contributed by 14p sequences.

Development and validation of a chromosome 15 DNA microarray for comparative genomic hybridization and application to autism. *T. Sahoo, C.A. Shaw, W. Yu, A.L. Beaudet.* Dept Human & Molec Gen, Baylor Col Medicine, Houston, TX.

Array based comparative genomic hybridization (CGH) provides a high resolution genome-wide scan for identifying DNA copy number variations. We are developing a high density large-clone based DNA microarray for detection of abnormalities involving chromosome 15. Additionally, we are implementing a robust CGH analytical tool for accurate and reproducible interpretation of genomic copy-number gains and losses. Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are known to map to 15q11-q13, a region which includes a 3-4 Mb domain subject to genomic imprinting. Paternal and maternal deletions of 15q11-q13 cause PWS and AS respectively. Cytogenetic abnormalities of chromosome 15q11-q13 also cause autism. Interstitial duplications and isodicentric 15q found in association with autism are usually of maternal origin suggesting both a gene-dosage effect and involvement of genomic imprinting. An initial version of our chromosome 15 microarray consisting of ~100 BAC-PAC clones provides a genome scanning resolution of better than one clone per Mb across chromosome 15 with the highest density across the PWS/AS interval and previously identified regions of instability on chromosome 15. Inclusion of subtelomeric clones for all other autosomes and a set of clones specific for chromosomes X and Y permits detection of cryptic unbalanced rearrangements. Initial validation of this technology involves specificity and sensitivity of detection using cytogenetically identified 15q rearrangements in autistic disorder. Chromosome 15-specific microarray CGH is enabling accurate identification of the whole spectrum of abnormalities (segmental gains and losses) seen in autism spectrum disorders and Prader-Willi/Angelman syndrome. Accurate prediction of the extent and boundaries of recurrent rearrangements in these disorders is providing clues towards an understanding of the mechanism leading to these rearrangements. Continuing development of array CGH will help identify deletions, duplications, and unbalanced structural rearrangements contributing to autism and provides a more cost-effective and sensitive approach to a molecular karyotype.

An unusual familial centromeric rearrangement involving chromosomes ?14/22 and 15 - A rare variant or translocation? *M. Sathanoori*^{1, 2}, *C. Minyon*¹, *M. Zimmer*³, *U. Surti*^{1, 2}. 1) Magee-Womens Hospital, Pittsburgh, PA; 2) University of Pittsburgh, Pittsburgh, PA; 3) Children's Hospital of Pittsburgh, Pittsburgh, PA.

A 2 y.o female child with hypotonia and developmental delay was referred for cytogenetic workup to R/O Prader-Willi Syndrome. Conventional cytogenetic analysis revealed a normal karyotype at 625 G-band level. FISH using the SNRPN probe (which includes D15Z1 and PML probe) revealed two normal signals for SNRPN and only one signal for D15Z1. Subsequent FISH analysis was performed with -satellite probes of acrocentric chromosomes (DZ13/21; DZ14/22; D15Z and D15Z4) and D15S11. The results are indicated in the table below. The finding of extra signal with DZ14/22 in both the mother and the proband indicates that this is a rare familial rearrangement. D15Z1 cross-hybridizes with #14 in 10-15% of individuals in the general population. A literature search failed to detect any variant #15 with absence of D15Z and presence of DZ14/22. Since our case has a 14/22 centromere on #15, it is a matter of debate as to whether it should be considered a variant #15 or a cryptic t(?14/22;15). A recent paper by Cockwell et al (2003) discusses the clinical significance of cryptic pericentromeric rearrangement and pregnancy outcome in couples with recurrent pregnancy loss. In addition to cryptic telomeric rearrangements, attention should be given to possible cryptic centromeric rearrangements.

Probe	D15Z1	D15Z	D15Z4	D15S11	SNRPN	PML	14/22	13/21
Locus	p11.2	p11-q11	p11-q11	q11-q13	q11-q13	q22	p11-q11	p11-q11
Case	+	+	+	++	++	++	+++++	++++
Control	++	++	++	++	++	++	++++	++++

'+' indicates the number of signals seen for the respective probe.

Male infertility associated with a familial translocation : t(1;13)(q24;q10). A. Sazci¹, N. Ercelen², E. Ergul¹, G. Akpinar¹. 1) Dept Medical Biol & Genetics, Kocaeli Univ, Kocaeli, Turkey; 2) V.K.V American Hospital, Department of Genetics, Guzelbahce sk. No:20, 34365, Nisantasi, Istanbul, Turkey.

Chromosomal rearrangements are a well-recognised cause of reproductive failure in males, with a significantly high rate of cytogenetic anomalies observed in azoospermia (13.7%). We report the case of a family with one sister and one brother in which the brother inherited a t(1;13)(q24;q10) translocation from his mother. The case with a clear translocation between the long arm of the chromosome 1 and the long arm of chromosome 13 t(1;13) (q24;q10) is described. The karyotype of the case was 46,XY,t(1;13)(q24;q10). Father and sister had normal karyotypes. We performed cytogenetic analysis and fluorescence in situ hybridization (FISH) for definition of chromosomal breakpoints. The translocation was found in a phenotypically normal male at the age of 38 who was born in Bulgaria from Turkish parents, and married for 9 years. Histological examination of testicular biopsies revealed spermatogenic failure. Although he had undergone bilateral varicocelectomy, his spermatogenic failure was not improved. Microdeletion analysis at the azoospermia locus (AZFa,b,c and d) of the Y chromosome revealed no deletions, thus suggesting another mechanism causing infertility in this translocated carrier. Molecular analysis of the family for atherothrombotic genes indicated that the proband and sister had a MTHFR T677T/A1298A compound genotype, while mother had a APOE4/4 genotype. In conclusion, this is the first case showing the t(1;13) (q24;q10) translocation involved in infertility in a male carrier but not in a female carrier.

Xp22.3 DELETION IN A GIRL ASSOCIATED WITH AUTISM, MENTAL RETARDATION AND MILD DYSMORPHIC FEATURES. *F. Scaglia, A. Patel, S. Peters, P. Panichkul, M. Shinawi.* Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

Males who are nullisomic for the Xp22.3 chromosomal region exhibit a phenotype consistent with the loss of one or more of the genes located in this region. Females with similar Xp deletions seldom manifest the same phenotype. It has been reported previously that *de novo* chromosomal deletions of Xp22.3 were found in three unrelated autistic females. Mutations in *Neurologin-4(NLGN4)*, located at Xp22.3, were found in two autistic brothers and in a large family with members affected by X-linked mental retardation, with or without autism, suggesting that this gene could be involved in autistic disorders. Here we describe a 10-year-old girl with an interstitial deletion of Xp22.3 who presented with mild dysmorphic features, autistic behavior and mild mental retardation. Her physical examination revealed a bulbous nose, hypertelorism, small ears, and a short, webbed neck. A brain MRI showed a mildly hypoplastic cerebellar vermis. Results of the Autism Diagnostic Observation Schedule and the Autism Diagnostic Interview Revised revealed that she exceeded the cutoffs for autism on both instruments. A chromosome analysis was subsequently carried out on peripheral blood lymphocytes from the proband and a *de novo*, cytogenetically visible deletion was detected at Xp22.31-p22.13. Results of detailed FISH analysis revealed that the telomeric breakpoint was localized between markers DXS8090 (4Mb) and DXS1056 (5.8Mb) and the centromeric breakpoint is between markers DXS1067 (8.9Mb) and DXS1043 (10.5Mb), so that the maximum size of the deletion is 6.5Mb. Of particular interest is the inclusion in this critical region of the *NLGN4* and the variably charged, X chromosome mRNA on CRI-S232A (*VCX-A*) genes that were recently implicated in autism and X-linked mental retardation, respectively. Other molecular studies are underway to analyze the parental origin of the abnormality, the X-inactivation pattern, and the epigenetic control of these genes. We propose that functional nullisomy, with or without skewed X-inactivation, in certain brain structures, or X-inactivation-independent haploinsufficiency for these genes can contribute to autism and mental retardation.

Detection of submicroscopic chromosome aberrations in children with idiopathic mental retardation by array-

CGH. *J. Schoumans¹, C. Ruivenkamp², A. Nordgren¹, AC. Thuresson³, E. Holmberg⁴, M. Nordenskjold¹, BM.*

Anderlid¹. 1) Dept of Molecular Medicine, Karolinska Institute, Stockholm, Sweden; 2) Dept of Human and Clinical Genetics, LUMC, Leiden, The Netherlands; 3) Dept of Clinical Genetics, Uppsala University Childrens Hospital, Uppsala, Sweden; 4) Department of Clinical Genetics, Sahlgrenska University Hospital/East Gothenburg, Sweden.

Novel high-resolution, whole-genome technologies, such as array based comparative genomic hybridization (array-CGH), improves the detection rate of submicroscopic chromosomal abnormalities. This opens new opportunities to re-investigate cases where conventional cytogenetic techniques, such as G-banding, FISH and metaphase CGH failed to detect any underlying genetic cause. We performed a high resolution genome-wide screening using array CGH on 40 patients with idiopathic mental retardation and dysmorphic features, in order to detect submicroscopic chromosomal rearrangements. We used a commercially available microarray from Spectral Genomics, containing 2600 BAC clones spaced at approximately 1 Mb intervals across the genome. Standard chromosomes analysis (450 bands) and multi-subtelomeric FISH screening was normal in all cases. We detected two interstitial deletions on 2q23-24 and 2q24-31 (size estimated 10 and 14 Mb respectively), one interstitial deletion on 4p16 (size estimated 7 Mb) and one terminal duplication on 9pter (size estimated < 1Mb). The deletions on 2q were confirmed by FISH, the duplication on 9p was confirmed by MLPA and confirmation of the 4p deletion is ongoing. Further investigation of parental samples revealed that both 2q deletions and the 9p duplication occurred de novo. The deletions on 2q were both visible by high resolution G-band analysis (>550 bands) but not by standard chromosome analysis (450 bands). The 9p duplication was not detectable by G-banding nor by subtelomeric FISH. In summary, we detected chromosome imbalances in 10% (4 out of 40) of the investigated cases. We therefore conclude that array CGH is an important tool for detection of submicroscopic rearrangements and is a promising technique for the future diagnostic setting.

Refinements in multiplex ligation-dependent probe amplification (MLPA) yield a robust, cost-efficient, high-throughput technology to assess genomic imbalance in pediatric populations. *R. Schultz^{1,4}, C. Duncan¹, F. Elder^{1,4}, K. Wilson^{1,4}, C. Read^{2,4}, N. Garcia^{3,5}, L. McDaniel¹.* 1) Dept Pathology, Univ Texas SW Medical Ctr, Dallas, TX; 2) Dept Ob/Gyn, Univ Texas SW Medical Ctr, Dallas, TX; 3) Dept Pediatrics, Univ Texas SW Medical Ctr, Dallas, TX; 4) McDermott Center, Univ Texas SW Medical Ctr, Dallas, TX; 5) Childrens Hospital of Dallas, Dallas, TX.

Conventional cytogenetics and fluorescence in situ hybridization (FISH) offer valuable tools to evaluate selected newborn populations; although some tests (e.g., the subtelomeric FISH panel) are fairly labor-intensive and expensive. MLPA offers another approach to detect unbalanced chromosomal alterations. This previously described assay employs hybridization of oligonucleotide (oligo) pairs to immediately adjacent genomic DNA sequences, with subsequent ligation and PCR amplification. The use of common flanking sequences, common primers and uniquely sized spacers for all oligo pairs facilitates multiplexing >40 targeted pairs in a single reaction. We have refined MLPA to establish a single, cost effective, and high throughput assay that can be readily applied to pediatric specimens using oligos targeted to all chromosomal ends and specific internal loci. Reactions are run on an ABI3100 and fragments analyzed with GeneMapper software. Analysis has been revised to evaluate peak area relative to an internal control with results expressed as a percent of total fluorescence for a specimen. The data are reproducible with small deviation permitting automated identification of abnormal results. Reconstruction experiments demonstrate reliable detection of mosaicism down to 25%. Screening specimens submitted for multiple congenital anomalies or developmental delay has identified various genomic abnormalities including deletions of 1p36, 18q21-qter, 13pter, and 5pter, as well as trisomies. The technology has high-throughput capabilities and is being developed as a general screen for appropriate populations. As a research tool, a revised oligo set has been devised to screen infants with diaphragmatic hernia for microdeletions in previously implicated chromosomal intervals.

Non-recurrent 17p11.2 deletions are generated by homologous and non-homologous mechanisms. *C.J. Shaw¹, J.R. Lupski^{1,2,3}*. 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Dept Pediatrics, Baylor Col Medicine, Houston, TX; 3) Texas Children's Hospital, Houston, TX.

Several recurrent, common chromosomal deletion and duplication breakpoints have been localized to large, highly homologous low copy repeats (LCRs). A well characterized example of this is the genomic interval 17p11.2-p12, wherein recurrent deletions and their reciprocal duplications result in hereditary neuropathy with liability to pressure palsies (HNPP), Charcot-Marie-Tooth disease type 1A (CMT1A), Smith-Magenis syndrome (SMS), or dup(17) (p11.2p11.2) syndrome. The mechanism responsible for these rearrangements, non-allelic homologous recombination (NAHR) between LCR copies, has been well established. However, fewer studies have examined the mechanisms responsible for non-recurrent rearrangements with non-homologous breakpoint regions. Here we analyze four uncommon deletions of 17p11.2, involving the SMS region. Using somatic cell hybrid lines created from patient lymphoblasts, we utilize a PCR-based strategy to refine the deletion breakpoints and to obtain sequence data at the deletion junction. Our analyses revealed that two of the four deletions are a product of Alu/Alu recombination, while the remaining two deletions result from a non-homologous end joining (NHEJ) mechanism. Of the breakpoints studied, 3/8 are located in LCRs, and 5/8 are within repetitive elements, including Alu and MER5B sequences. These findings suggest that higher-order genomic architecture, such as LCRs, and smaller repetitive sequences, such as Alu elements, can mediate chromosomal deletions via homologous and non-homologous mechanisms. These data further implicate homologous recombination as the predominant mechanism of deletion formation in this genomic interval.

BAC probes with FISH delineates a 6q interstitial deletion in an individual with a Prader-Willi like phenotype. *S. Shetty, J. Lauzon, M. Innes, J. Chernos.* Department of Medical Genetics, University of Calgary, Alberta Children's Hospital, Calgary, Alberta, Canada.

Prader-Willi syndrome (PWS) is associated with obesity, hyperphagia, hypotonia and developmental delay. Many individuals present with a similar constellation of findings and are found not to have the typical molecular and cytogenetic findings of PWS. Different conditions including other chromosomal abnormalities have been associated with a PWS-like phenotype such as interstitial deletions of 6q. The SIM1 gene is mapped to 6q16.3-q21 region. It has been proposed as a candidate gene for obesity and may play a role in the phenotype of these individuals.

We report a case of an individual with a PWS-like phenotype with small interstitial deletion of the long arm of chromosome 6. This 23 year-old man was seen for evaluation of his learning disabilities and compulsive eating habits. He had hyperphagia and history of food-seeking behaviours. He had neonatal hypotonia and feeding problems. His weight increased significantly at the age of 8. Physical features included short palpebral fissures, full lips and brachydactyly with tapered fingers. Previous investigations included a normal muscle biopsy, Fragile X and PWS methylation testing.

Cytogenetic analysis at 500-525 band resolution revealed a karyotype of 46,XY, del(6)(q14q16). In order to delineate the breakpoints precisely, molecular cytogenetic studies were performed using directly labelled BAC probes with FISH, which showed an interstitial deletion of 6q16 region.

To our knowledge this is the first case of an individual with a 6q interstitial deletion and a PWS-like phenotype to be diagnosed by a molecular cytogenetic approach. BAC probes for the 6q16 region were useful in identifying the apparent deletion of the SIM1 gene region in this patient. Using directly labelled BAC probes with FISH is a reliable and inexpensive approach for delineating interstitial deletions. This technology may provide an economical and efficient adjunct to the cytogenetic studies of patients with PWS like phenotype and normal PWS methylation or 15q11.2 FISH studies.

Mosaic whole-arm duplication of the long arm of chromosome 1 identified in an amniocentesis and confirmed in different tissue samples at birth. *P.L. Sinanaj¹, A.E. Deupree¹, E. Williamson¹, E. Varga², S.A. Berend¹.* 1) Genzyme Genetics, Santa Fe, NM; 2) University of Kansas Medical Center, Kansas City, KS.

Chromosome 1 is the largest human chromosome and contains approximately 10% of the genome. Full trisomy of the long arm of chromosome 1 results in multiple, major anomalies and is not compatible with survival to term. Mosaic trisomy 1q is also rare; previous reports have described multiple anomalies in affected fetuses including diaphragmatic hernia, hypoplastic lungs, and cleft palate. We report a case involving a patient referred for amniocentesis due to hydrocephalus, ureteropelvic junction (UPJ) obstruction, and an atrial-septal defect identified on ultrasound. Cytogenetic analysis using standard GTG banding techniques revealed a mosaic chromosome pattern with fifteen colonies (15/18, 83%) showing a direct duplication of the long arm of chromosome 1 (q21.1q43). The remaining three colonies showed a normal female chromosome constitution. It was unclear if the three colonies containing normal female cells represented maternal cell contamination indicating a fetus with full trisomy for 1q or if this represented true fetal mosaicism. Parental chromosome analyses indicated that this was a de novo rearrangement. The baby was delivered at 30 weeks gestation and died approximately one hour after birth. Cord blood and tissue samples were obtained and sent for confirmation of the prenatal analysis. Chromosome analysis was performed on each of the tissue samples and the abnormal cell population was confirmed in all tissue types and found in different proportions: cord blood (6/104, 6%), skin (1/30, 3%), muscle (1/30, 3%), and tendon (17/30, 57%). Interestingly, the proportion of abnormal cells varied greatly in the different tissue types.

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An Automated Platform for Quantitative FISH Analysis. *F. Tafas, M.W. Kilpatrick, I. Ichetovkin, A. Seppo, Y. Kim, Y. Agarwall, P. Tsipouras.* Ikonisys Inc, New Haven, CT.

In the course of the last decade, FISH analysis has emerged as a standard of care in clinical testing. However, FISH is expensive, labor intensive, requires a high skill level and can be difficult to interpret. A fully automated system for FISH analysis would, therefore, be of great value for the clinical testing laboratory. The Ikoniscope™ is a customized system developed specifically for cell identification and analysis. Epi-fluorescence optics and a slide management system provide high speed and accuracy of slide movement. Fast focusing is achieved with the assistance of an infrared-laser device. Slides are fed to the stage by an automated slide/cassette feeder allowing unattended processing of 175 slides. The system is specifically designed for automated microscopy and is capable of operating in a networked fashion in large clinical laboratory settings on a 24/7 basis. Image capture is performed through a monochrome CCD camera offering high sensitivity and resolution as well as high-speed image acquisition. Prior to slide processing an exposure setting procedure ascertains proper signal intensity for acquisition and processing. Highly optimized image analysis algorithms provide automated counting of the number of FISH signals for each chromosome. The viewer software component of the system displays 10x images of all fields scanned, and 100x images of nuclei and the FISH signals within them for all nuclei automatically chosen for high magnification analysis. These results are displayed, along with the automatically determined FISH signal quantitation, and an interactive set of tools to assist the cytogeneticist in confirming the number of FISH signals present. In a preliminary study of 30 slides prepared for conventional interphase FISH analysis of amniocentesis samples, the system demonstrated its potential for providing FISH counting results highly correlated with fully manual assessment. In addition to improving the ease of undertaking FISH based assays and the quality of data generated, this platform has the potential to improve both the sensitivity of FISH analysis and the accuracy of the result produced.

9p subtelomere deletion: pathogenic mutation or normal variant? *C. Techakittiroj¹, K. Kim¹, H. Andersson^{1,2}, J. Thoene^{1,2}, M. Li^{1,2}.* 1) Hayward Genetics Ctr, Tulane Univ Medical Sch, New Orleans, LA; 2) Departments of Pediatrics, Tulane University Health Science Center, New Orleans.

FISH using chromosome-arm-specific subtelomere probes has proven to be a powerful tool in identifying cryptic chromosome rearrangements among patients with multiple congenital anomalies (MCA) and/or mental retardation of unknown causes. We report a patient with MCA and an inherited deletion of 9p subtelomeric region. The patient was a newborn boy with prenatal history of polyhydramios, cystic hygroma, and IUGR. At birth, he was noted to have a flat face, bilateral anotia, skin pits and tags along the mandible, webbed neck, undescended left testis, arachnodactyly, bilateral arthrogryposis, meconium ileus and peritonitis. Echocardiogram revealed multiple cardiac anomalies including type two truncus arteriosus and total anomalous pulmonary venous return. MRI showed abnormal brain development and fluid accumulation in the posterior fossa. High resolution chromosome studies displayed a normal male karyotype: 46, XY; however, subtelomere FISH studies revealed deletion of 9p subtelomeric region. Parental chromosome studies appeared normal; though, the mother, who is phenotypically normal, was found by subtelomere FISH to carry the same deletion as the baby. The 9p subtelomere deletion thus may not be responsible for the phenotype of the baby. Alternatively, the 9p subtelomere deletion could be responsible for the babys phenotype if further deletion and/or duplication had occurred during the transmission of the 9p subtelomere deletion from the mother to the child. Another possible cause of the childs MCA might be loss of heterozygosity due to an autosomal recessive gene mutation at the paternal 9p subtelomeric region. Molecular studies comparing the 9p subtelomere deletion in the mother and in the child are needed to further illustrate the etiology of the MCA in this patient.

Isolated ambiguous genitalia with 46,XY,del(11)p13 karyotype. *L. Telvi*¹, *C. Bouvattier*², *C. Nezelof*¹, *J.P. Barbet*¹, *P. Bougneres*². 1) Cytogenetics Laboratory, Hopital St Vincent de Paul, Paris, France; 2) Pediatrics Department, Hopital St Vincent de Paul, Paris, France.

We report a child with 46,XY,del(11)(p13)(RHG)(RTBG) karyotype. He showed ambiguous genitalia and no ocular, no renal abnormalities. The patient was born on 7.22.1987, to non consanguineous parents after 40.5 weeks of gestation. The birth length and weight were 46.5 cm and 2600 g respectively. At birth, the patient presented with a 18x10 mm genital bud, fused and striated labioscrotal folds, bilateral cryptorchidism and no palpable gonads, no uterus, no vagina. The SRY gene was found present after FISH technique. At five months, the patient was operated for feminizing genitoplasty. Two small (10x8x4 mm) testis was taken and found histologically normal. The patient presented retarded psychomotor development with an IQ = 73. No mutation was found for androgen receptor (exons 1&8). The parents and the older sister and brother were clinically normal. The WT1 gene located at chromosome 11p13 is required for normal genitourinary system. Mutations of the WT1 gene usually leads to WAGR syndrome, Denys-Drash syndrome or Frasier syndrome. Mental retardation, genitourinary, ocular and renal abnormalities or gonadoblastoma are frequently associated with these syndromes. This patient presented with a deletion at 11p13 site showed isolated genitourinary anomalies. We compare this patient to all reported cases.

Dual Structural Alterations in a Case with Angelman Syndrome. *J.H. Tepperberg, H. Waters, I. Gadi, S. Griffin, L. Wisniewski, P.R. Papenhausen.* Dept Cytogenetics, Laboratory Corp of America, Res Triangle Pk, NC.

We report a cryptic complex karyotype in a 1.2 year old female referred with non-specific developmental delay. Chromosome analysis showed a supernumerary ring-like marker chromosome in 16 of 21 metaphases examined. The remaining cells appeared to be normal. Parental studies are pending. Ring chromosomes are generally mitotically unstable, as is in this case, and the amount of euchromatin can vary considerably. Although euchromatin origin can usually be ascertained, prognosis is often difficult. A Multiprobe alpha satellite pericentromeric FISH device (CytoCell, Inc.) was used to identify the marker chromosome and showed that the marker chromosome was derived from chromosome 15. Additional FISH to rule out a trisomy or tetrasomy dosage effect using a SNRPN DNA probe (15q11.2), showed that the supernumerary chromosome 15 was SNRPN negative. Surprisingly, one of the chromosome 15 homologues showed a chromosome 15q11.2 SNRPN deletion in all cells examined. Reverse transcription PCR showed that the SNRPN transcript was present, and is thus consistent with a diagnosis of Angelman syndrome. The ring chromosome, to our knowledge appears to be benign. A plausible theory is that a meiotic I unequal exchange between the homologues of the maternal chromosome 15 resulted in the formation of a duplication 15q on one homologue and a deletion on the second. Subsequently, ring formation occurred from the duplicated chromosome, with breakage proximal to SNRPN, and concomitant non-disjunction. This would ultimately result in the observed chromosome complement.

An XX male carrier of the SRY gene with skewed inactivation of the normal X chromosome. *M.A. Thomas^{1,2}, A.M.V. Duncan^{2,3,4}, R. Fetni^{3,4}.* 1) F. Clarke Fraser Clinical Genetics Unit, Division of Medical Genetics, Department of Pediatrics, Montreal Children's Hospital, Montreal, Quebec, Canada; 2) Department of Human Genetics, McGill University, Montreal, Quebec, Canada; 3) Department of Pathology, Montreal Children's Hospital, Montreal, Quebec, Canada; 4) Department of Pathology, McGill University, Montreal, Quebec, Canada.

Introduction: One cause of XX maleness is the presence of the testis determining SRY gene on one of the X chromosomes, due to a recombination between the X and Y chromosomes during paternal meiosis. Ninety percent of these individuals have normal male external genitalia, however, some have ambiguous genitalia or are true hermaphrodites. Differential inactivation of the X chromosome carrying SRY and the size of the translocated SRY region have been suggested as the major mechanisms for this phenotypic variability. **Case:** We present a 36-year-old male who was found to have azoospermia during an evaluation for infertility. Hormonal testing demonstrated hypergonadotropic hypogonadism. Chromosomal analysis showed a 46, XX karyotype. FISH studies confirmed the presence of SRY in the terminal part of Xp. The X-inactivation pattern was analyzed in 50 lymphocytes by replication studies, combined with FISH, and showed more than 90% of the normal X was late replicating, and therefore, inactivated. **Discussion:** Although the SRY gene plays a critical role in male differentiation, there are XX male carriers of SRY who have ambiguous genitalia or are true hermaphrodites. This has raised the question of whether there is another factor that contributes to male differentiation in XX males. Skewed inactivation of the X chromosome carrying the translocated SRY gene has been found in true hermaphrodites and individuals with ambiguous genitalia. Our case demonstrates a male with normal external genitalia who has skewed inactivation of the normal X, thus allowing sufficient SRY expression for male differentiation. This assumes that the gonadal cells demonstrate the same distribution of X-inactivation as lymphocytes.

Phenotype and origin of two subtelomeric deletion/duplication of 1q/1p and 16p/16q. *F. Tihy, J. Michaud, E. Lemyre.* Dept Genetics, Hosp Ste-Justine, Montreal, PQ, Canada.

Duplication/deletion chromosomal rearrangements are often described as the result of meiotic recombination in the gametes of pericentric inversion carriers. With the use of sub-telomeric probes in fluorescent in situ hybridization, cryptic dup/del rearrangements can now be detected.

We diagnosed two such cryptic dup/del rearrangements: one involving the subtelomeric regions of chromosome 1 and the other of chromosome 16. Those particular rearrangements and their phenotypes have not yet been described to our knowledge. In the first case, a subtelomeric dup 1p/del 1q was diagnosed in a boy with moderate developmental delay, microcephaly, corpus callosum hypoplasia, hypopituitarism, pre-auricular pit, bifid uvula, aplasia cutis, an ASD, and a VSD. The mother carried a semi-cryptic inversion involving both subtelomeric regions. She previously had a phenotypically normal son. With the length of chromosome 1, there is probably as much chance to have an even number of crossing over resulting in a balanced gamete or an odd number of crossing over leading to an unbalanced gamete. The second patient was diagnosed with a del 16p/dup 16q. His phenotype involved moderate mental retardation, hypertelorism, Pierre Robin sequence, laryngomalacia, ASD, primary arterial pulmonary hypertension and C2-C3 subluxation. This rearrangement occurred de novo and was not the recombinant of an inversion.

In both cases the findings of the cryptic del/dup allowed proper genetic counselling for the families.

De novo isodicentric X chromosome: 46,X,idic(X)(q24). A. Tsai¹, C. Rawlinson¹, C. Walton¹, L. McGavran², J.P. Johnson³. 1) Div Clinical Gen & Metabolism, Childrens Hosp, Denver, CO; 2) Colorado Genetics Laboratory, Denver, CO.; 3) Shodair Childrens Hospital Genetics Laboratory, Helena, MT.

We present a 9-year-old patient with a de novo isodicentric X chromosome, karyotype of 46,X,idic(X)(q24), noted to have normal growth and development, and no dysmorphic features. Comparison of the proband with previously reported patients with an isodicentric X chromosome, of various breakpoints in Xq, showed that many patients have characteristic features as a result of the isodicentric X chromosome. These include a range from normal phenotype, the lack of secondary sexual characteristics development, secondary amenorrhea, and primary amenorrhea with unpredictable ages of onset. The X-inactivation study on peripheral blood revealed 99% inactivation of the isodicentric X chromosome. This patient demonstrates the absence of unusual physical characteristics associated with an isodicentric X chromosome in late childhood which has not been reported before, as well as the importance and need for further characterization by molecular and cytogenetic studies in patients with an isodicentric X chromosome.

Unbalanced 16;22 translocation leading to multiple anomalies and the -thalassemia trait in an Acadian patient. F.

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Alpha-thalassemia occurs predominantly in populations of Mediterranean, African, or Southeast Asian descent. There are two duplicate copies of the α -globin gene located on the short arm of each chromosome 16, for a total of four α -globin loci. Patients with α -thalassemia trait are typically asymptomatic and have deletion of two α -globin genes. In the Mediterranean and Southeast Asian populations the deletions are usually in *cis* while in the African American population, the deletions are typically in *trans*. We present a 3-year old Southwestern Louisiana Acadian patient with a history of transient anemia, mild synophrous, prominent forehead, downslanting palpebral fissures, bowed upper lip, ectrodactyly and syndactyly of the toes, atrial septal defect, seizures, and developmental delay. Chromosomes appeared normal. Southern blot revealed a deletion of two α -thalassemia genes in *cis*. Fluorescent *in situ* hybridization (FISH) with subtelomere probes showed the presence of a derivative 16 chromosome. The der(16) resulted from a translocation between 16p and 22q, causing monosomy for the 16p13.3 and trisomy for 22q13.3. Previous reports of 16p subtelomere deletions have shown minimal phenotypic effects. Since our patient's translocation involves the 16p subtelomere, it is likely that his multiple anomalies are due to trisomy in the 22q subtelomere region. Phenotype/cytogenetic correlations in previous reports of 22q trisomy have usually involved a larger portion of the 22q, and the overlapping effects from the other involved chromosome, making it difficult to delineate a specific phenotype associated with trisomy for this region. Further reports of trisomy for the 22q subtelomere region should help determine if there is a consistent phenotype. Patients who are found to have α -thalassemia trait due to deletion of two α -globin genes in *cis*, but are not a member of an ethnic group in which this typically occurs, should have molecular and cytogenetic evaluations including subtelomeric FISH, especially if multiple anomalies and developmental delay are also present.

Partial trisomy of 16q and partial trisomy of Xq in a girl with 47,XX,+der(X)t(X;16)(q10;q10?). *C. Uria Gomez*^{1,2}, *G. Arteaga Ontiveros*², *E. Blanco Aguirre*³, *M.J. Gonzalez Gutierrez*². 1) Dept Genetics, Inst Natl de Perinatologia, Mexico, Mexico; 2) Genecon Diagnostico Laboratorio, Toluca Mexico; 3) Hospital General Adolfo Lopez Mateos, Toluca Mexico.

Partial trisomy of chromosome 16q is an uncommon chromosome disorder, and association with partial trisomy Xq is a rare event. Patients with only partial trisomy 16q show dysmorphic features and multiple congenital anomalies such as distinctive facies, mental retardation, vertebral, genital and anal abnormalities, cleft palate, renal anomalies and camptodactyly. X inactivation is random but in cases of carriers of unbalanced X;autosome translocation the abnormal chromosome is almost always inactivated. We present a two months-old girl who was referred to us for cytogenetic analysis because she presented dysmorphic features. Chromosomal analysis in peripheral blood lymphocytes with GTG bands showed 47,XX,+der(X)t(X;16)(q10;q10?) in all the metaphases. Parental chromosomes appeared normal. Derivative chromosome was further confirmed with C-bands and FISH using whole chromosome paints for 16 and X. Additionally we use centromeric probe for the X, and a signal in the derivative chromosome and in both X chromosomes was observed. This is an unusual chromosome abnormality. To our knowledge there are no reports about an association between partial trisomy 16q and Xq. We discuss the cytogenetic findings and the abnormal phenotype, but further studies to determine the role of X inactivation on the resulting phenotype as well as association of 16q trisomy in this patient will be necessary to characterize phenotype-karyotype correlation.

Position effects due to chromosome breakpoint mapping ~1 Mb upstream and ~1.3 Mb downstream of *SOX9* in two cases with Campomelic dysplasia. *G.V.N. Velagaleti*^{1,2}, *G. Bien-Willner*³, *J.K. Northup*², *L.H. Lockhart*¹, *S.M. Jalal*⁶, *J.R. Lupski*^{3,4,5}, *P. Stankiewicz*³. 1) Depts. Pediatrics, and; 2) Pathology, Univ Texas of Medical Branch, Galveston, TX; 3) Depts. Molecular & Human Genetics, and; 4) Pediatrics, Baylor College of Medicine, Houston, TX; 5) Texas Children's Hospital, Houston, TX; 6) Dept. Pathology, Mayo Clinic, Rochester, MN.

Campomelic dysplasia (CD) is a semilethal skeletal malformation syndrome with or without XY sex reversal. In addition to multiple mutations within the *SOX9* gene on 17q24.3, several chromosome translocations and inversions with breakpoints scattered over 1 Mb upstream to *SOX9* leading to haploinsufficiency have been described (Pfeifer et al. 1999). Here, we present an apparently balanced translocation t(4;17)(q28.3;q24.3), segregating in a family with a mild acampomelic CD and Robin sequence. Both breakpoints have been identified by FISH within single BAC clones. The 17q breakpoint maps ~ 0.9 Mb upstream of *SOX9*, which is within the same BAC clone as in the previously reported case. A somatic cell hybrid has been generated in our case and the breakpoint is being currently sequenced. DNA sequence analysis of the 17q breakpoint revealed a region conserved in human, chimpanzee, and mouse and candidate transcripts have been identified. Interestingly, Jamshidi et al. (2004) reported an isolated Robin sequence in a family segregating with a balanced translocation t(2;17)(q24.1;q24.3). The 17q breakpoint in this family maps very close to our breakpoint. We also report a prenatal identification of acampomelic CD with a male to female sex reversal in a fetus with a de novo apparently balanced complex karyotype 46,XY,t(4;7;8;17)(4qter->4p15.2::17q25->17qter;7qter->7p15::4p15.2->4pter;8pter->8q12.2::7p21.2->7pter;17pter->17q25::8q12.2->8qter). All breakpoints have been mapped within single BAC clones. Surprisingly, the 17q breakpoint maps ~1.3 Mb downstream of *SOX9*. This is the first report of CD with the chromosome breakpoint mapping distal to *SOX9*. We discuss the possible molecular mechanisms responsible for the position effect.

Sequencing of the breakpoint junction fragments of an X;autosome translocation in a female with Duchenne muscular dystrophy. *A.M. Vianna-Morgante, I.E. Kerkis, A.C.V. Krepischi-Santos.* Departamento de Biologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil.

We sequenced the breakpoints of a de novo balanced translocation $t(X;22)(p21.1;q13.1)$, ascertained through a female with Duchenne muscular dystrophy (DMD), aiming at disclosing the mechanism originating the rearrangement. The derivative X chromosome was of paternal origin and was shown to be always active. Breakpoint mapping was performed by FISH and DNA analyses in the patients cells and in somatic cell hybrids. The X chromosome breakpoint was mapped to intron 47 in the *DMD* gene (Xp21.1), contained in BAC RP11-607K23. The *DMD* gene disruption in association with the inactivation of the normal X led to the dystrophy phenotype. Chromosome 22 breakpoint was mapped to a sequence cloned in BAC RP11-60G6, and then located between genes *GRAP2* and *BC031099*. The junction fragments of both derivative chromosomes were cloned by PCR using primers constructed with reference to chromosome X and 22 sequences flanking each mapped breakpoint. The exchange sites on chromosomes X and 22 have in common two adjacent base pairs (GC). Comparison of the junction sites with the sequences on the normal chromosomes revealed the loss of six base pairs, four from chromosome 22 as well as two of the shared GC pairs. The two remaining GC pairs are on the der(22). Nonhomologous end-joining then appears as the mechanism originating this translocation. (This study was supported by FAPESP and CNPq).

Analysis of an add(21)(q22.2) characterized as der(21)t(1;21)(q42.1;q22.2) by fluorescence *in situ* hybridization in a patient with mental retardation and primary amenorrhea. D.T. Walsh¹, K.H. Ramesh¹, V.R. Pulijaal¹, M. Zohouri¹, R.A. Conte¹, D. Wei¹, T. Zhou¹, A.R. Deshikar¹, R. Freeman², L.A. Cannizzaro¹. 1) Department of Pathology, Montefiore Medical Center, Bronx, NY; 2) Department of Ob/Gyn, Montefiore Medical Center/Albert Einstein College of Medicine, Bronx, NY.

Chromosomal studies were conducted on a 30 year old patient presenting with primary amenorrhea. Other clinical features include mental retardation; IQ of 38, the patient is nonverbal but responsive to verbal commands. Additionally, a systolic heart murmur was detected and endocrinological testing revealed elevated gonadotropin levels, consistent with ovarian failure. A pelvic ultrasound indicated a very small uterus and underdeveloped ovaries. High-resolution peripheral blood chromosomal analysis revealed an abnormal female karyotype, with an abnormal chromosome 21 having additional chromosomal material of unknown origin at band q22.2. This additional material was further characterized by fluorescence *in situ* hybridization (FISH) as being derived from the terminal end of the long arm of chromosome 1 (1q42.1-1qter). FISH and GTG results indicate the karyotype of this patient to be: 46,XX,add(21)(q22.2).ish der(21)t(1;21)(q42.1;q22.2)(wcp1+,D1S378+,wcp21+,VIJyRM2029-). The patient is trisomic for 1q42.1-1qter and monosomic for 21q22.2-21qter regions. Confirmation of the der(21), whether it was de novo or due to malsegregation of a parental t(1;21)(q42.1;q22.2) could not be established, as her parents were not available for cytogenetic evaluation. Primary amenorrhea has often been linked with X chromosome anomalies, especially X-autosome translocations, indicating a role played by genes present in the X chromosome in the development of ovaries. This study and other reported autosomal translocations support the idea that the genes present in autosomes may also play a role in gonadal development.

Array-CGH characterization of an inversion duplication of chromosome 5p in a patient with congenital defects.

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Inverted intrachromosomal duplications of chromosome 8p and 2q have been well characterized. In contrast, inverted duplication of chromosome 5p has rarely been reported and the causing mechanism is unknown. A 4-year-old boy with language and motor skill delay was studied. He initially presented as a premature newborn at 32.5 weeks with congenital anomalies including hypotonia, failure to thrive, seizures, and pre-auricular skin tags. The parents were non-consanguineous and there was no contributing family history. Karyotyping revealed a de novo rearranged short arm of chromosome 5. FISH analysis using subtelomeric and cri-du-chat critical region probes demonstrated a deletion in the subtelomeric region, while the cri-du-chat region (5p15.2) was duplicated. Comparative Genomic Hybridization (CGH) showed a gain in the 5p14-5p15.2 region and a probable loss within 5p15.3. Sub-Megabase Resolution Tiling-set (SMRT) array-CGH (aCGH) was used to further characterize this rearrangement. The deleted region spanned from the telomeric region to the distal part of 5p15.31, while the duplication concerned the proximal region of band 5p15.31 to the distal end of 5p14.3. This result was further proven using FISH with BAC probes mapping to the breakpoint regions. This study illustrates the power of aCGH for the characterization of chromosomal rearrangements. In a one-step experiment, we were able to narrow down the deleted and duplicated regions of chromosome 5p, and to locate the breakpoints within a region covered by a small number of BACs, thus allowing further FISH experiments for precise location. These results were consistent with inversion-duplication occurring in this chromosomal region. Using the human genome data, this approach will allow rapid identification of genes potentially responsible for the phenotype. Further characterization of the breakpoints may also uncover underlying genomic features, such as duplicons, explaining the occurrence of this rearrangement.

Prenatal diagnosis of a fetus affected with Down syndrome and deletion 1p36 syndrome by fluorescence in situ hybridization and spectral karyotyping. *B. Wang¹, L-J. Hsieh¹, T-C. Hsieh², G-P. Yeh², M-I. Lin¹, M. Chen^{1,2,3}.* 1) Ctr Medical Genetics, Changhua Christian Hosp, Changhua, Taiwan; 2) Dept. Obstetrics and Gynecology, Changhua Christian Hosp, Changhua, Taiwan; 3) Dept. Obstetrics and Gynecology, National Taiwan University Hospital, Taiwan.

A fetus having partial trisomy of the distal part of chromosome 21q due to a de novo translocation is reported here. A 29-year-old woman received amniocentesis at 18 weeks of gestation because of abnormal ultrasound findings including bilateral choroid plexus cysts, atrioventricular septal defects, rocker-bottom feet, and possible hydrocephalus. Cytogenetic analysis revealed 46,XY, add (1)(p36.3), in which an additional material of unknown origin was attached to one of the terminal short arm of chromosome 1. Parental blood studies showed normal karyotypes in both parents. Spectral karyotyping (SKY) was then performed and the origin of the additional material locating at chromosome 1p was found to be from chromosome 21. Conventional FISH analysis was also used and confirmed the SKY findings by use of a chromosome 21 specific painting probe, a locus specific probe localized within bands 21q22.13-q22.2 and a 21q subtelomeric probe. A hidden Down syndrome caused by a de novo translocation in this fetus was therefore diagnosed and the karyotype was designated as 46,XY, der(1)t(1;21)(p36.3;q22.1) .ish der(1)(WCP21+, LSI 21+, 1pTEL-, 21q TEL+) de novo. Clinical features of the 1p36 deletion syndrome are also reviewed and may contribute to some features of this fetus. Termination of pregnancy was performed at 20 weeks of gestation. To our knowledge, our case appears to be the first case having partial monosomy 1p and partial trisomy 21q caused by de novo translocation being diagnosed prenatally.

Molecular delineation of two chromosomal abnormalities in autism. *T.H. Wassink¹, V.C. Sheffield^{2,3}, J. Piven⁴, S.R. Patil².* 1) Dept Psychiatry, Carver Coll Med, Univ Iowa, Iowa City, IA; 2) Dept Pediatrics, Carver Coll Med, Univ Iowa, Iowa City, IA; 3) Howard Hughes Medical Institute; 4) Dept Psychiatry, Univ North Carolina, Chapel Hill, NC.

Autism is attributable to chromosomal abnormalities in at least 5% of cases; we describe here two such cases. Case 1 is a male, the older of two affected siblings. He has mild mental retardation, impaired fine motor skills, and slightly dysmorphic facial features (wide-set eyes and depressed nasal bridge). His language development was delayed, his social activity is limited, he has circumscribed interests, and he engages in verbal rituals and compulsions. His younger sibling has more severe language impairment and social dysfunction, and his mother experienced language delay and problems with articulation. The patient was found through participation in a linkage study to have maternally derived disomy of chromosome 1. Follow-up genotyping of densely spaced polymorphic markers showed that the chromosome was primarily heterodisomic except for two regions of isodisomy attributable to recombination. Case 2 is a singleton male with autism found to have an interstitial 13q deletion [46,XY,del(13)(q33.2q34)]. This patient experienced significant delays in the development of language and social behavior. His articulation is poor, his vocabulary limited, and his expressive language has not yet progressed beyond single words. The extent of the deletion was narrowed using polymorphic markers to a 7 MB interval near the end of 13q. The relationships of these abnormalities to other findings in autism, and additional candidate genes that they suggest, are discussed.

Trisomy 8 mosaicism, a new case of the old syndrome. *J. Watts¹, M. Janes¹, J. Xu².* 1) Pediatrics; 2) Pathology and Molecular Medicine and Lab Medicine, Hamilton Health Sciences and McMaster University, Canada.

A male infant was born at 26 weeks gestation, weight 810 gm. Fetal ascites and abdominal calcification were present on antenatal ultrasound. Cytogenetics analysis of cultured blood taken at age of 6 days showed a trisomy 8 mosaicism; 47,XY,+8[7]/46,XY[23]. The cultured cells of the skin tissue from abdomen at 3 months and 17 days revealed trisomy 8 in all 30 cells examined. The cultured blood cells taken at age of 5 months and 24 days showed a normal male karyotype in all 30 cultured cells. He developed intestinal obstruction due to malrotation requiring a Ladd procedure, complicated by necrotising enterocolitis, and prolonged feeding problems necessitating gastro-jejunal feeds. Gastric emptying was virtually absent. He was dysmorphic with long ears, dysplastic pinnae with bilateral superior points and small external auditory meatus, a small pointed nose, clinodactyly of both 5th fingers and hypoplastic toenails. Partial agenesis of the corpus callosum, left-ventricular non-compaction, an atonic bladder and cataracts were present. As a consequence of prematurity, he had broncho-pulmonary dysplasia, intraventricular hemorrhage leading to a porencephalic cystic, and detached retinae from retinopathy of prematurity. Hypothyroidism was present, possibly due to prematurity. Multiple episodes of sepsis occurred. While clinical findings of trisomy 8 syndrome have been documented in more than 100 cases, dysmotility as found in the present case is not commonly described. Although surgical events may have played a role, its severity in this case together with the bladder atonia, suggests an underlying neuromuscular functional abnormality as a consequence of the chromosomal anomaly. In addition, ventricular non-compaction is a new finding, and may account for cardiac failure in previous cases where major anatomic defects were absent.

An unbalanced translocation involving 15q11.2 in a phenotypically normal individual. *L. Wisniewski, P. Papenhausen, J. Tepperberg, I. Gadi, P. Singh-Kahlon, E. Cantu.* LabCorp, Research Triangle Park, NC.

A 34 year old phenotypically normal male was referred for chromosome analysis due to a history of recurrent miscarriage. GTW banding revealed an unbalanced translocation between the long arm of chromosome 15 and the short arm of chromosome 20, 45,XY,-15,der(20)t(15;20)(q11.2;p13). There was no evidence of mosaicism for the balanced form. C banding confirmed that the derivative was monocentric. The G banding pattern clearly suggested loss of material from bands 15q11.2 and/or 20p13. Absence of clinical abnormalities associated with Prader-Willi/Angelman syndromes lead to the investigation of distal 20p with the subtelomere probe D20S1157(Vysis, Inc.). However, a normal 20p subtelomere signal was evident, prompting the conclusion that the 20p breakpoint was localized in the telomere sequences. Examination of proximal 15q with SNRPN and D15S10 revealed that these loci were also intact. Presumably, the material deleted due to the translocation was confined to the short arm, centromere and proximal pseudogene segment proximal to SNRPN. Duplication of this material has previously been reported as having no clinical effect. This case illustrates the importance of fully characterizing unusual rearrangements with available flanking FISH markers before finalizing the interpretation of their clinical interpretation. Unbalanced structural rearrangements involving proximal 15q are frequently associated with Prader-Willi/Angelman syndromes due to partial deletions. However, assessing band 15q11.2 visually with conventional banding is problematic. In the present case, the patient's phenotype clearly did not support the expected phenotype/karyotype correlation of a known microdeletion syndrome. The alternative clinical interpretation, that of mental retardation/developmental delay due to a presumed terminal deletion of 20p, was also shown to be incorrect by the results of subtelomere FISH.

Molecular cytogenetics characterization of trisomy 13/trisomy 18 mixoploidy in a boy with moderate clinical features. *J. Xu*^{1,2}, *T. Heshka*^{2,3}, *D. Whelan*^{1,2}, *J-X. Cui*⁴, *F-Y. Han*⁴, *Y-G. Xie*⁴. 1) Pathology & Molecular Medicine; 2) Lab Medicine; 3) Pediatrics, Hamilton Health Sciences and McMaster University, Hamilton; 4) Lab Medicine, Memorial University, Newfoundland, Canada.

We present a case with trisomy 13/trisomy 18 mosaicism in a newborn baby boy. The proband was born at 40 3/7 weeks of gestation to a 25 year old G2T2L2. The patient has postaxial polydactyly bilaterally of the hands and of the right lateral toe. The patient also has a prominent nasal bridge, hypertelorism, epicanthic folds, low set ears, a right abducted thumb and hypospadias. Ultrasounds of the head, spine and kidneys were normal. An echocardiogram revealed a patent ductus arteriosus. The patient is presently 4 years old. He can walk with a walker and drink from an open cup. His height and weight are below the 3rd centile. Cytogenetics study of peripheral blood revealed an abnormal male karyotype in all cells; 47, XY,+13[9]/47,XY,+18[21]. This double trisomy mosaicism was confirmed in a repeat blood sample showing 47,XY,+13[15]/47,XY,+18[40] and a skin tissue showing 47,XY,+13[22]/47,XY,+18[28]. The parents have a normal karyotype. A preliminary analysis of DNA polymorphism shows that the extra chromosome 13 was resulted from maternal meiosis I nondisjunction. Further study is underway to determine a) the parental origin and stage of nondisjunction for the extra chromosome 18; and b) mosaicism vs. chimera. This is the 5th case of trisomy 13/trisomy 18 mixoploidy and is characterized by moderate clinical features. The mother went on to have a normal male child. We hypothesize that the mix of these two trisomies might help alleviate lethal effects from each individual trisomy, which might account for the finding of moderate clinical features in our patient.

Opitz Trignocephaly C syndrome in a patient with a 9q34.3 monosomy: phenotype variation by deletion size.

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We identified five unrelated patients with 9q34.3 deletions, including one patient with the clinical diagnosis of Opitz Trignocephaly C syndrome (OTCS). Several individuals with 9q34.3 deletions have been reported recently in association with recognizable facial dysmorphism, mental retardation, speech delay, hypotonia, microcephaly, and early childhood obesity. OTCS is a rare and believed to be autosomal recessive disorder but of unknown etiology; characterized by trigonocephaly, flat, broad nasal bridge with the short nose, an abnormal deeply furrowed palate, polysyndactyly, short limbs, hip dislocation, heart defects, cryptorchidism, hypotonia, and midline brain anomalies. The patients described herein have *de novo* terminal 9q34.3 deletions detected by high-resolution cytogenetic and/or molecular analyses. We mapped the deletions size within overlapping BAC clones by FISH analysis. These deletions showed heterogeneity in the location of the breakpoints and variation in size. We found that, dysmorphic features, **hypotonia, obesity, microcephaly**, delay or absence of expressive speech (HOMS syndrome) observed in the 9q34.3 deletion are characteristic and present in four of our patients, regardless of deletion size. Cardiac defects, seizures, limb and brain anomalies have been detected in patients with larger deletions. The patient with the OTCS has been found to have the largest deletion and the most severe phenotype. Our results suggest that OTCS may represent a complex phenotype due to haploinsufficiency of several genes within chromosome 9q34.3. We propose that 9q34.3 monosomy results in either HOMS syndrome or OTCS depending on the extent of deletion. Critical regions and candidate genes for this newly defined contiguous gene syndrome are discussed.

Congenital anomalies in an infant with a small interstitial deletion on the short arm of chromosome 7. A.

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The patient was a female fraternal twin delivered at 33 weeks gestation to a 40 year old G3P2 mother. The male twin was unremarkable, however the patient presented with intrauterine growth retardation, mild dysmorphic features, and a two-vessel umbilical cord. Chromosome analysis of peripheral blood cells revealed 46,XX, del(7)(p14.3p15.2). Chromosome 7 painting did not support a complex rearrangement or translocation. Postnatal evaluation revealed a patent ductus arteriosus, bilateral vesicoureteral reflux, and failure to thrive. At 7 weeks of age, her growth parameters were at the 50th percentile for 34 weeks gestation. Dysmorphic features included microcephaly, patent anterior and posterior fontanelles, flat facial profile with mild frontal bossing, short palpebral fissures and periorbital fullness, upturned nares with flat nasal bridge, smooth philtrum, small lowset pointed ears with prominent antihelix, small mouth and chin with slightly highly-arched palate, umbilical hernia, and slightly prominent clitoris. Thumbs were proximally placed with decreased flexion of the interphalangeal joints; toes 2-4 showed partial syndactyly, were proximally placed, and had a hammertoe configuration. A CT study of the skull failed to demonstrate craniosynostosis.

Five cases have been reported in the literature with deletions of 7p13p15. This case highlights an exceptionally small deletion of 7p which shares a few clinical findings with other patients carrying larger 7p deletions: growth and developmental delay, proximal insertion of thumbs, syndactyly of toes, and absence of significant cardiac or skull anomalies (skull anomalies are observed only in distal deletions of 7p). We propose that the critical region for the common clinical findings we observed lies within 7p14.3 to p15.2.

Pathogenesis of the trisomy 16 placenta. *P.J. Yong*¹, *D.E. McFadden*², *C.D. MacCalman*³, *W.P. Robinson*⁴. 1) MD/PhD Program; 2) Departments of Pathology; 3) Obstetrics and Gynaecology; 4) and Medical Genetics, University of British Columbia, Vancouver, BC.

Trisomy 16 (t16) is the most common trisomy in spontaneous abortion (SA), and is associated with IUGR and preeclampsia (PE) when confined to the placenta in ongoing pregnancies. SA, IUGR and PE have been associated with poor uterine invasion by extravillous trophoblast (EVT) from placental chorionic villi (CV). Thus t16 EVT may be abnormal.

Placentas were obtained from SAs 6-13 wks gestation. Since SAs undergo tissue degeneration before expulsion or surgery, tissue culture was performed. CV explants were cultured on Matrigel, and EVT confirmed by immunohistochemistry (IC). EVT outgrowths from the explants were observed in only 5/20 cases 10 wks, but in 17/30 cases 10 wks ($p = 0.015$). For those cases 10 wks, EVT outgrowths occurred in 7/9 euploid (eu) cases compared to 0/4 t16 cases ($p = 0.021$). Since t16 EVT did not grow, protein expression was assessed in placental fibroblasts from 4 eu cases and 3 t16 cases. Fibroblasts were cultured from collagenase-digested CV explants, with maternal contamination and other cell types excluded by PCR and IC. Protein was extracted at the 3rd passage at 75% confluence, and the expression of 75 protein kinases profiled using a 2D Western blot (Kinexus Inc.). Five kinases (CDK1, CDK7, PKC-, S6K p70, IKK-) were underexpressed, and 2 kinases (PKG1, ERK1) overexpressed, in the t16 cases compared to the eu cases ($p < 0.05$). There was no confounding by gestational age. Of the 3 kinases with genes on chromosome 16 (CK2, PKC-, ERK1), only ERK1 was overexpressed in the t16 cases (~2x higher). A phosphoantibody to T202/Y204 showed ~3x higher levels of phosphorylated (activated) ERK1. In addition, the coefficient of variation (V) of each of the 75 kinases was calculated for the t16 and eu cases: greater variability in protein expression was seen among the t16 cases (mean V: 0.51 vs. 0.12, $p = 0.0015$).

In conclusion, a defect in EVT outgrowth and altered protein kinase expression may be implicated in the pathogenesis of the t16 placenta.

Mosaic X/XX/XXX male with atypical Klinefelter features. C.W. Yu¹, J. Sistrunk², Y. Yang¹, C. Thompson¹, C. Friedrich¹. 1) Dept Preventive Medicine, Univ Mississippi Medical Center; 2) Baptist Medical Center, Jackson, MS.

The estimated incidence of XX males is approximately 1 in 20,000 males (de la Chapelle 1972, 1981; Nielsen and Sillesen 1975). Several hypotheses have been offered to explain the male phenotype in the absence of a normal Y-chromosome in these males. We report a case of mosaic X/XX/XXX male with some features of Klinefelter syndrome and the causes of mosaicism. A 34-year-old white male was referred for infertility study. He had a total testosterone of 289 ng/dL, FSH of 34.5 mIU/ml, LH of 14.9 mIU/ml, and ACTH of 35 picograms/ml. Other than notable childhood asthma, there are no known genetic disorders in his family. His sperm counts showed azospermia and/or minimal sperm present. G-banded blood chromosomes revealed that 42 cells had 46 chromosomes with two Xs, 6 cells had 45 chromosomes with one X, and 2 cells had 47 chromosomes with three Xs. FISH was performed using LSI probes for SRY and Kallmann and alpha satellite DNA probes for X and Y centromeres. For the metaphases with 46 chromosomes, the SRY locus was present only in one of the X chromosomes while Kallmann and DXZ1 were seen in both X chromosomes. For the metaphases with 45 or 47 chromosomes, one copy of SRY, Kallmann and DXZ1, and one copy of SRY and three copies of Kallmann and DXZ1 were seen respectively. No DYZ1 locus was detected in any of the metaphases examined. Parental chromosome study was not available. Cytogenetic and FISH studies revealed that this patient has a normal X and a derivative X chromosome, resulting from a translocation of a partial Yp to the Xp terminal, in a majority of the cells. The breakpoint is around p11.3 for the Y and posterior to the Kallmann locus (p22.3) for the X. Furthermore, a non-disjunction of the normal X chromosome occurred during the somatic cell division to form the 45,X and 47,XXX cell lines. Thus the karyotype for this patient is: 46,X,der(X)t(X;Y) (p22.3;p11.3)/ 45,idem,-X/ 47,idem,+X. The presence of partial Yp material after the X/Y interchange accompanied by the somatic cell non-disjunction and non-random X inactivation in those cells with two or more X chromosomes probably accounts for the atypical features of the Klinefelter syndrome.

A patient with two isodicentric Y chromosomes and radioulnar synostosis. *A.L. Zaslav¹, J. Jacob¹, R. Kazi¹, D. Blumenthal², J. Fox².* 1) Dept Laboratory Medicine, Long Island Jewish Medical Ctr, New Hyde Park, NY; 2) Dept of Peds, Schneider Childrens Hosp, The Albert Einstein College of Medicine, New Hyde Park, NY.

Tetrasomy for the Y chromosome is rare. To our knowledge, only three cases of non-mosaic tetrasomy for the Y have been reported. Here we present a case of non-mosaic tetrasomy Y in a one-month-old male. Echocardiogram was normal. X-ray revealed left radioulnar synostosis. CAT scan of the head showed mild prominence of the Sylvian fissures, frontal temporal subarachnoid space and mild ventriculomegaly. Physical examination revealed radioulnar synostosis, which has been previously reported in phenotypic males with sex chromosome abnormalities. Peripheral blood chromosome analysis of 50 cells with GTG and C-banding revealed a karyotype of 47,X, idic(Y)(q12)x2. This was confirmed by FISH using the DYZ3 Y centromere probe and the Yp11.3, SRY probe recognizing the SRY region of the Y chromosome (Vysis, Downers Grove, IL). The father had a normal karyotype.

To our knowledge, non-mosaic tetrasomy for the Y chromosome has been previously reported in only three cases. There is a wide range of clinical and phenotypic variability among patients with this karyotype. A review of other non-mosaic tetrasomy Y cases and possible correlation between karyotype and phenotype will be attempted. This report will be helpful in determining the phenotype of patients with this rare chromosome abnormality.

A boy with an apparently balanced three-way translocation t(5;8;17) and autism. *L. Zwaigenbaum¹, T. Heshka^{1, 2}, J. Xu².* 1) Pediatrics; 2) Pathology and Molecular Medicine and Lab Medicine, Hamilton Health Sciences and McMaster University, Canada.

We report a 3 year and 8 month old boy with autism and a de novo, apparently balanced complex chromosome rearrangement. He was born following an uncomplicated pregnancy at 37 weeks gestation with a birthweight of 1880g (< 3rd percentile), and required initial support for respiratory distress, hypoglycemia, and feeding difficulties. All developmental milestones were delayed. He first walked at age 2 years, and remains essentially non-verbal at age 5 years. He has poor eye contact, lacks functional communication skills, does not participate in social interaction, and exhibits stereotyped behaviors and repetitive play interests. He meets criteria for a diagnosis of autistic disorder, based on structured clinical history using DSM-IV criteria, and assessment using the Autism Diagnostic Observation Scale at age 3 years. Physical examination did not identify any dysmorphic features. Notably, his younger brother has global developmental delay but not autism. Analysis by G-banding in combination with FISH and SKY revealed that the proband had an apparently balanced 3-way translocation t(5;8;17)(q31.1;q24.1;q21.3). Possibility of microdeletions in Angelman syndrome critical region in 15q11-q13 was ruled out by FISH using probes (GABRB3 and D15S10) and DNA methylation studies. The parents and his brother all had a normal karyotype by routine G-banding. The breakpoints 8q24.1 and 17q21 have been previously reported to be implicated in autistic spectrum disorder (ASD) and autism, respectively. Our case suggests that rearrangements at 5q31.1, 8q24.1 and/or 17q21.3 may predispose to ASD in addition to other clinical features.

Replication timing of different regions of the Y chromosome: A study by FISH using BACs. *S. Dubé^{1, 3}, R. Fetni⁴, N. Lemieux^{1, 2, 3}*. 1) Département de pathologie et biologie cellulaire, Université de Montréal, Montréal, Canada; 2) Département de pathologie, Hôpital Sainte-Justine, Montréal, Canada; 3) Centre de recherche de l'Hôpital Sainte-Justine, Montréal, Canada; 4) Department of pathology of the Montreal Children's Hospital, University McGill, Montreal, Canada.

Recent studies have showed that replication timing, i.e. the time when a locus is replicated, reflects gene expression. A gene which replicates early in S phase will be expressed in the cell, whereas a late replicating gene will not be expressed. Also, actively expressed genes seem to have a loose chromatin structure as opposed to inactive genes that show a more condensed chromatin organization. To date, few loci have been studied according to these two aspects. We hypothesized that a correlation between replication timing and chromatin condensation exists. The first objective of this study is to determine the replication timing of different regions of the Y chromosome. To reach this goal, we are using a FISH replication assay and bacterial artificial chromosomes (BACs) probes covering the Y chromosome. This variant FISH technique can determine the replication time of a DNA sequence in interphase nuclei of unsynchronized normal lymphocytes. An early replicating sequence shows a high percentage of double-dot signals, while a late replicating sequence mostly displays single-dot signals. So far, sequences mapping to bands Yq11.21 and Yq11.222 have been studied and showed a late replication timing. Therefore, these sequences would not be expressed in normal lymphocyte cells. With this project, in addition to determine the replication timing of important genes and regions of the Y chromosome, we want to establish the existence of replication domains and correlate these data with gene expression. Furthermore, we will study the possible link between replication time zones and chromatin organization by analyzing differential compaction of those sequences on the Y chromosome. Consequently, we will be able to see if chromatin structure and replication timing have an influence on the occurrence of preferential DNA rearrangements on the Y chromosome. *Supported by RMGA-FRSQ and the Fondation de l'Hôpital Sainte-Justine.*

Characterization of maternally derived aberration of chromosome 15q in a patient with autism. *D.A. Kwasnicka¹, W. Roberts², M. Li¹, S. Russell¹, S. Choufani¹, S. Scherer^{1, 3}.* 1) Department of Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, ON, Canada; 2) Department of Pediatrics, The Child Developmental Center, Toronto, ON, Canada; 3) Department of Molecular and Medical Genetics, University of Toronto, Ontario, Canada.

Autism is a severe neurodevelopmental disorder characterized by deficits in social interaction, disturbed communication and stereotype routines and interests. Evidence from twin and family studies supports the idea of a strong genetic etiology. Autism has been found in association with other medical conditions such as fragile X syndrome, phenylketonuria, Williams-Beuren syndrome, or neurofibromatosis type 1. Cytogenetic abnormalities in the Prader-Willi/Angelman syndrome (PWS/AS) critical region (15q11-13) have also been described in individuals with autism. Observations of maternal duplications affecting chromosome 15q11-13 in patients with autism and evidence for linkage disequilibrium in this region in autism families indicate the existence of a susceptibility locus. In this study we describe a 6-year-old girl diagnosed with severe autism, overall developmental delay, delayed expressive, and receptive language. The karyotype was designated 47, XX, idic(15)(q13). Parental karyotype was normal. Fluorescence in situ hybridization (FISH) using bacterial artificial chromosome (BAC) clones spanning the long arm of chromosome 15 showed that the patient has additional copy of 15q11-13 region. Molecular analysis of the proband and the parents using polymorphic markers within 15q11-q13 region confirmed FISH results and determined that the extra chromosome 15 material was maternal in origin. Duplication of the 15q11-13 segment represents the most consistent abnormality reported in association with autism. This case report reinforces the hypothesis that additional copies of this chromosome segment may be causally related to autism.

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Advanced Paternal Age Affects Recombination. *S.E. Schrump, T.J. Hassold.* Genetics, Case Western Reserve, Cleveland, OH.

Recombination between homologous chromosomes during meiosis I is required for proper chromosome segregation. In the absence of recombination or in situations where recombination is altered, chromosomes may nondisjoin, resulting in aneuploidy. We and others have been interested in knowing whether this factor is linked to the other agents known to be associated with aneuploidy, i.e. increasing parental age. Specifically, does the level of meiotic recombination change with age? While some information on this question is available from humans, data sets are small and the results contradictory. Thus, as a first step toward understanding this relationship, we have initiated studies of recombination and increasing age in the male mouse. Using a novel assay to detect recombination events, we examined the number of exchange events in males of three inbred strains, C57BL/BJ, Spret/EiJ, DBA/2J, in three age groups, young (2-5 months), middle aged (10-13 months), and old (18 months). Preliminary studies indicate a slight, yet significant increase in recombination with increasing age. Likewise, there is a slight increase in the variation of recombination events in these animals with increasing age. While preliminary, these data provide the first evidence to indicate a breakdown in the meiosis I checkpoint as males get older.

Measurement of chromosome specific telomere length on chromatin/DNA fibers. *J. Yan, E. Bouchard, B.Z. Chen, R. Drouin.* Dept Pediatrics/Medical Genetics, CHUS, Sherbrooke, PQ, Canada.

Human telomeres consist of tandem repeats of TTAGGG sequence that extends from 2 to 15 kb. The length of telomeric sequence plays important roles in maintaining chromosome stability. A number of attempts have been made by many investigators to measure the telomere length in different normal and cancer populations. The relative telomere length has been calculated either from certain cell lines in an overall manner using terminal restriction fragments (TRF) analysis or from the measurement of the fluorescence signal intensity on telomeres using quantitative FISH (Q-FISH) technique. However, the TRF does not yield information on individual chromosome ends, and the restriction enzymes used in Southern blot cleave at sites in the subtelomeric DNA that are at variable distance from the beginning of actual TTAGGG repeats. The Q-FISH approach is based on the calculations of the signal intensity of fluorescence, for which the measurement would rely on the hybridization efficiency and would be more or less affected by uneven fluorescence lightness in each single field under the microscope. We have developed a fiber-FISH technique for the direct measurement of individual telomere length. The PNA probe labeled with green for the telomere sequence and the probe specific for the 1q subtelomere sequence labeled with red, which serves as physical length reference, were co-hybridized on the chromatin/DNA fiber preparations. The results showed that the length of measurable telomere and subtelomere signals aligned on such fibers ranged from 0.9-2.9 μ m and 13.8-29 μ m, respectively, according to the stretching degree of the fibers. It implies that 3.4-7.2 Kb/ μ m (average 5.5 Kb/ μ m) of the physical length of the probes hybridized on such fibers. Using this technique, we demonstrated that the mean telomere length in the 1q from a lymphoblast cell line is 8.4 Kb with SD 1.9 Kb. This reflects a precise physical length for a particular telomere and can be used as a reference to determine all other telomere lengths that are measured from metaphase chromosomes by Q-FISH.

Hot spots for ectopic recombination in hominoid evolution: evidence from comparative cytogenetics of human chromosome 3. *Y. Yue*¹, *B. Grossmann*¹, *E. Tsend-Ayush*², *F. Gruetzner*², *M. Ferguson-Smith*³, *F. Yang*³, *T. Haaf*¹. 1) Inst Human Genetics, Mainz Univ Sch Med, Mainz, Germany; 2) Comparative Genomics Group, Australian National Univ, Canberra, Australia; 3) Centre for Veterinary Science, Cambridge Univ, Cambridge, UK.

Comparative FISH mapping of some 150 large-insert clones revealed at least 14 independent evolutionary breakpoints between human chromosome 3 and the genomes of four representative primates. Many rearrangements, i.e. the pericentric inversions leading to orangutan 2, were not simple breakage and reunion events, but involved microduplications and/or microdeletions. The breakpoint regions between human 3 and orangutan 2 contain paralogous sequence blocks, which were duplicated at many subtelomeric regions during hominoid evolution. FISH of fully integrated BAC maps to orangutan, siamang gibbon and silvered-leaf monkey chromosomes combined with precise breakpoint localization by PCR analysis of flow-sorted chromosomes localized three independent rearrangements of the human 3q21.3-syntenic region within a 230 kb BAC contig. Approximately 200 kb of the human 3q21.3 segment were not present on the homologous primate chromosomes, suggesting a genomic DNA insertion into the breakpoint region in the lineage leading to humans and African great apes. The inserted segment represents part of an ancestral duplication. The 3q21.3-syntenic breakpoints in the orangutan and siamang genomes were narrowed down to <5 kb and <13 kb DNA segments, which are enriched with endogenous retrovirus LTRs and other repetitive elements. The three breakpoint regions between human 3p25.1, 3p12.3, 3q21.3 and orangutan 2 as well as their paralogous segments represent breaks of chromosomal synteny between the human, mouse, rat and/or chicken genomes. Collectively our data suggest reuse of short recombinogenic sites in primate and mammalian evolution. Large-scale chromosome rearrangements, microduplications and microdeletions can be considered as different aspects of an inherent instability of these regions. Evidently, genome architecture, in particular low-copy repeats have played an important role in shaping mammalian genomes.

A simple and rapid method of direct CVS preparation for interphase *FISH*. *S. Bahieg*¹, *L. Edelmann*¹, *B. Levy*^{1,2}, *N.B. Kardon*^{1,2}. 1) Department of Human Genetics, Mount Sinai School of Medicine, New York, NY; 2) Department of Pediatrics, Mount Sinai School of Medicine, New York, NY.

In recent years, fluorescence in situ hybridization (*FISH*) on interphase nuclei prepared from fetal cells has been implemented in most cytogenetic laboratories. Laboratories perform this testing most often in response to abnormal ultrasound findings and/or serum screening results or in cases of isolated parental anxiety. Therefore, the assays must be performed without delay and as quickly as possible to ensure the rapid report of results. We developed a simple method for preparing interphase nuclei from small amounts (1-2 mg) of direct CVS. A 50% glacial acetic acid solution was used as an initial dissociating agent, eliminating the need for lengthy collagenase digestion. The dissociated cells were then microfuged at high speed to free the nuclei from the cellular debris followed by fixation in methanol/glacial acetic acid (3:1) with a minimum of three successive fixative changes. Slides were prepared and AneuVysion (Vysis) probes were applied to the nuclei and hybridizations were carried out for 30 minutes and two hours with the X,Y,18 probe set and 13,21 probe set, respectively. This protocol requires 4 hours from receipt of the sample to report of the results. To date, this method has been used in our laboratory to process 104 CVS specimens with 12 aneuploidies detected and 100% concordance with results obtained from GTG banded karyotypes of cultured CVS. Thus, this new method of direct CVS preparation for interphase *FISH* offers a very rapid, inexpensive and highly accurate alternative to traditional methods.

Comparative genomic hybridization microarray analysis: diagnostic considerations to detect common ploidy and sex chromosome anomalies. *B.C. Ballif¹, C.D. Kashork¹, L. Patterson³, R. Saleki¹, E. Rorem¹, K. Sundin¹, B.A. Bejjani^{1,2,3}, L.G. Shaffer^{1,2,3}.* 1) Signature Genomic Laboratories, Spokane, WA; 2) Health Research and Education Center, Washington State University, Spokane, WA; 3) Sacred Heart Medical Center, Spokane, WA.

Chromosome analysis is vital to the understanding of the causes of fetal demise and multiple miscarriages. Autosomal trisomy occurs in ~52% of miscarriages with abnormal karyotypes. Triploidy is the next most frequent anomaly, occurring in ~19% of chromosomally abnormal miscarriages. We recently developed a microarray that contains contigs of three or more BAC clones for 41 subtelomeric regions, 43 pericentromeric regions and 41 known microdeletion syndromes. This microarray is ideal for detecting aneuploidy and unbalanced chromosomes. Among 39 cases with abnormal karyotypes used for validation of our microarray, 11 were derived from products of conception (POC), including seven trisomies, one 45,X, two multiple aneuploidies, and one triploidy with tetrasomy 8. All cases were hybridized to two microarrays using a dye-reversal strategy. In a blinded study, all autosomal single copy gains (trisomies) and losses (45,X) were correctly detected. The microarray did not accurately detect the gain of the X chromosome in a 49,XXX,+2,+15 and only detected the gain of chromosome 8 in the triploidy. During the analysis, we hybridized the DNA from the POCs with DNA from an opposite sex normal control. In doing so, we could not discriminate a trisomy X from a normal XX female, when a normal XY male was used as the control. One component to the analysis of the data is the normalization of control to patient sample ratios. In the case of triploidy, the abnormal 3:2 gain across the genome affects the normalization process by forcing the DNA ratio to 1:1. Therefore, to accurately identify sex chromosome aneuploidies, we have implemented the use of a cell line from a Klinefelter male, 47,XXY, as our control for all microarray analyses. Although the analysis of triploids remain a diagnostic challenge for genomic microarrays, this experience was essential to understand the complexities in analyzing unknown samples in the clinical setting.

Identification of the reciprocal duplication of the NPHP1 gene that causes nephronophthisis. *H. Baris*¹, *B.A. Bejjani*², *B. Ballif*², *C.D. Kashork*², *R. Saleki*², *E. Rorem*², *K. Sundin*², *L.G. Shaffer*², *V.E. Kimonis*¹. 1) Genetics, Children's Hospital Boston, Boston, MA; 2) Signature Genomic Laboratories, Spokane, WA.

Many microdeletions that result in clinically recognizable syndromes are flanked by low copy repeat (LCR) sequences that mediate nonallelic homologous recombination. During this event in meiosis, for each resulting microdeletion, the reciprocal microduplication product is produced. For some microdeletion syndromes, such as DiGeorge syndrome and Smith-Magenis syndrome, the reciprocal duplications result in clinically distinct phenotypes. However, the reciprocal duplications have not been described for many of the remaining syndromes including nephronophthisis, caused by homozygous deletion of the NPHP1 gene at 2q13. Recently, this deletion has also been described in Joubert syndrome in which some patients also suffer from renal failure due to nephronophthisis. We identified an adopted individual with a single copy gain of the NPHP1 gene following microarray analysis using the SignatureChip. The duplication was confirmed with a BAC clone containing the NPHP1 gene. The boy was referred for microarray testing at 12 years of age because of pervasive developmental delay, attention-deficit hyperactivity disorder, obsessive compulsive disorder and behavioral problems. On physical examination we noted small testes of 2ml each with normal penile length, bilaterally adducted thumbs, and dysmorphic features consisting of synophrys and long palpebral fissures. A chromosome analysis and brain MRI were both normal. Ophthalmologic evaluation and renal ultrasound are in progress. This is the first identified case of NPHP1 duplication. It is unclear whether this duplication is related to the clinical phenotype in this patient. We anticipate that other duplications associated with the reciprocal microdeletions will be uncovered through the use of microarrays. Novel syndromes are likely to be identified, and the etiologies of known syndromes will be explained. Microarrays are proving to be a critical adjunct to clinical cytogenetics for identifying submicroscopic rearrangements of clinical importance.

Detection of cryptic deletions by genomic microarray in a patient with a complex four chromosome translocation. *B.J. Baty, J.C. Carey, Z. Chen, B. Issa, B.C. Cowley, E. Aston, T. Maxwell, A.R. Brothman.* Div Med Gen/Dept Pediatrics, Univ Utah Health Sciences Ctr, Salt Lake City, UT.

We report a patient studied as a newborn with prenatal growth deficiency of weight and mildly dysmorphic physical features. Cytogenetic studies revealed a *de novo* two-way, apparently balanced translocation between 6p and 11q, and a three-way translocation with 6q to 7p, 7p to 14q and 14q to 6q. An unrecognized chromosomal imbalance was hypothesized when the proband presented with developmental delay at 15 months. Growth was at the 2nd-5th centile. His only significant health problem was severe cyclic vomiting. Physical findings included bilateral epicanthal folds, low nasal bridge, ptosis, full lips, broad mouth, short fingers, clinodactyly, hypotonia, anal tag, myopia, low frontal hairline, neck webbing, and pectus carinatum. He developed ADHD, aggression, and preverbal behaviors. At 15 years, he was receiving special education services. The above interpretation was confirmed by chromosomal studies [46,XY,t(6;7;11;14)(11qter11q13.5::6p23q23.3::14q32.114qter); 6pter6p23::7p137qter;11pter11q13.5::6q25qter); 14p1314q32.1::6q23.36q25::7p137pter)]. FISH using whole chromosome paint probes helped characterize the specific regions involved in this complex rearrangement. Genomic microarray studies with a Spectral Genomics (Houston, TX) 1 Mb chip revealed deletions at 6p22.3 and 6q23.2, confirmed by FISH probes [nuc ish del(6)(p22.3p22.3)(RP1-130G2-),del(6)(q25.2q25.2)(RP11-535A9-)]. These deletions were close to the breakpoints identified in the initial karyotypic interpretation. Confirming the long suspected imbalance (i.e. deletions) proves the chromosomal basis of the syndrome and eliminates the need for further diagnostic testing. It also confirms the low recurrence risk for the parents, but poses high reproductive risk for the proband. This is an example of the utility of new technology in the characterization of a genetic abnormality. This case also underscores the importance of genomic microarray analysis in the evaluation of patients with suspected unbalanced abnormalities which cannot be discerned by conventional karyotyping.

Construction of a diagnostically useful microarray to identify chromosome imbalance. *B.A. Bejjani^{1,2,3}, C.D. Kashork³, R. Saleki³, E. Rorem³, K. Sundin³, B.C. Ballif³, L.G. Shaffer^{1,2,3}.* 1) Health Research and Education Center, Washington State University, Spokane, WA; 2) Sacred Heart Medical Center, Spokane, WA; 3) Signature Genomic Laboratories, Spokane, WA.

Chromosome analysis is an important component to the diagnosis of congenital anomalies, developmental delay and mental retardation. Routine chromosome analysis identifies aneuploidy and structural rearrangements >5 Mb. Array CGH was developed to increase the resolution of chromosomal studies by using large-insert clones as targets for analysis. We constructed a microarray for the clinical diagnosis of medically significant and relatively common chromosomal alterations. We chose 906 BAC clones for the 41 telomeric regions, 43 pericentromeric regions, and 41 known microdeletion loci. All BACs were evaluated by FISH mapping under constant conditions. FISH revealed that 7% of the clones were mismapped based on map locations obtained from two publicly available databases (58 mapped to the wrong chromosome and 3 mapped to a different locus on the same chromosome), 17% cross-hybridized to other chromosomes, and 10% did not hybridize or showed poor hybridization signals under uniform FISH conditions. Thus, of the 906 clones selected, only 589 (65%) were deemed adequate for arraying on this clinical device. For each clinically relevant locus, at least three overlapping BACs were selected. Additionally, for the microdeletion loci, BAC contigs flanking each locus were chosen. Each BAC was spotted four times on the microarray making it functionally equivalent to >2,000 FISH experiments. This work illustrates that the rigorous assessment of BACs and their use in array CGH is especially important when the microarray is used for clinical diagnosis. Additionally, a targeted array reduces the number of polymorphisms, uninformative or uninterpretable clones and is more appropriate for diagnostic use than whole-genome microarrays at this time. It is anticipated that genomic microarrays will be an important adjunct to the clinical molecular cytogenetic investigation of spontaneous abortions, newborns and children with mental retardation, developmental delay, and congenital anomalies.

Mother to son amplification of a small terminal deletion as a new potential mechanism of familial recurrence in microdeletion syndromes. *F. Faravelli¹, R. Lecce², M. Murdolo², G. Neri², F. Dagna Bricarelli¹, M. Di Rocco³, M. Zollino².* 1) Human Genetics, Ospedale Galliera, Genova, Italy; 2) Istituto di Genetica Medica, facolt di Medicina A. Gemelli, UCSC, Roma, Italy; 3) Pediatria II, Istituto Giannina Gaslini, Genova, Italy.

We report on a instance of familial recurrence of a microdeletion syndrome mediated by mother to son amplification of a small terminal deletion. Two brothers were affected by WHS. The first patient died at 18 years of age. He had a MCA/MR syndrome fully consistent with a WHS diagnosis. Karyotype was normal and molecular cytogenetic analysis was not performed. The youngest brother is now 17 years old. He suffers from severe growth retardation, cleft palate, hypospadias, typical facial appearance, severe mental retardation and seizures. Standard chromosomes were apparently normal. FISH analysis, performed when he was 11 years old, confirmed the diagnosis of WHS (46, XY.ish del (4p) (F26-; IS28-; D4S96-; D4S43-; D4S182-; D4S180 X 2). The deletion spanned about 3 Mb, including both WHS critical region (WHSCR) and WHS critical region 2 (WHSCR-2). The recurrence of phenotype between the two brothers led us to further investigations. A preliminary FISH analysis with the 33c6 probe (D4S43), deleted in the proband, excluded the presence of a cryptic translocation in both parents. A skin biopsy was performed in other to rule out mosaicism for the microdeletion. In this occasion a non mosaic smaller deletion was detected in the mother, spanning about 1.5 Mb from D4S96 to the telomere. Her karyotype was 46, XX.ish del(4p)(F26-; IS28-; D4S96-; FGFR3 X 2; D4S43 X 2). Both WHSCR and WHSCR-2 were fully preserved. Interestingly, she presented with some phenotypic abnormalities (facial dysmorphisms, mild mental impairment, febrile convulsions) but these were not fully consistent with a diagnosis of WHS. We describe the clinical phenotype of the mother in relationship to the small deletion and discuss this undescribed potential mechanism of recurrence of microdeletion syndromes.

Phenotypic description and molecular characterization using QMPSF of a subtelomeric 20p deletion. A.

Goldenberg¹, P. Saugier-Veber^{1, 2}, C. De La Rochebrochard¹, C. Magniette¹, G. Joly-Hélas³, E. Bessenay¹, S. Marret⁴, M. Tosi², T. Frebourg^{1, 2}. 1) Department of Genetics, Rouen University Hospital, 76031 Rouen, France; 2) Inserm U614, Faculty of Medicine, 22 boulevard Gambetta, 76183 Rouen, France; 3) Department of Cytogenetics, Rouen University Hospital, 76031 Rouen, France; 4) Department of Neonatology, Rouen University Hospital, 76031 Rouen, France.

Constitutional subtelomeric imbalances account for approximately 6 % of idiopathic mental retardation (MR). We have developed a new simple molecular genetics assay based on the QMPSF method (Quantitative Multiplex PCR of Short Fluorescent Fragments) to detect and map subtelomeric rearrangements. The telomeric QMPSF is based on the simultaneous amplification under quantitative conditions of short fluorescent genomic fragments specific of each subtelomeric end. Telomeric QMPSF analysis of patients with MR led us to identify a 20pter deletion in a 5 years old girl referred for MR and autistic features. She was the first child of unrelated parents. She walked unaided at the age of 23 months. At the age of 5 years, she presented with moderate mental retardation, social interaction deficiency and moderate facial dysmorphism. She had no malformations and normal growth. The 20pter deletion was also detected in her mother who presented a mild mental disability and similar facial dysmorphic features. This chromosomal imbalance was confirmed by FISH in the index case and her mother. The flexibility of the QMPSF method allowed the design of a new QMPSF, including 4 amplicons covering the 20pter region, and this new QMPSF revealed that this subtelomeric deletion was smaller than 2.3 Mb. This case report constitutes one of the rare descriptions of a 20pter deletion. Additional cases will be necessary to give further delineation of this recognizable phenotype.

Validation of a clinical microarray: identification of chromosome imbalance and polymorphisms. *C.D. Kashork¹, R. Saleki¹, E. Rorem¹, K. Sundin¹, B.C. Ballif^d, L.G. Shaffer^{1,2,3}, B.A. Bejjani^{1,2,3}.* 1) Signature Genomic Laboratories, Spokane, WA; 2) Health Research and Education Center, Washington State University, Spokane, WA; 3) Sacred Heart Medical Center, Spokane, WA.

We have constructed a microarray using 589 BACs covering the 41 telomeric regions, 43 pericentromeric regions, and 41 known microdeletion syndrome loci for use in clinical diagnosis of cytogenetic imbalances. In addition, we selected BACs that flank all of the microdeletion syndrome loci to use as controls for the consistent-sized deletions and to identify larger deletions. We expected this microarray to identify cytogenetic imbalance due to deletions, duplications, aneuploidies, and derivative chromosomes but not mosaicism. We tested this array in a set of blinded experiments on a cohort of 50 phenotypically normal individuals. Even after careful assessment of the BACs through FISH localization prior to constructing the microarray, the array identified deletion/duplication polymorphisms in the 100 normal chromosomes. The polymorphic loci included the pericentromeric regions of 2p, 7q, and 14q, and the subtelomeric regions of 5q, 6p, 14q, and 20p. All polymorphisms detected by the array were confirmed by FISH with a BAC from the appropriate locus. In addition to the analysis of 50 chromosomally normal individuals, we analyzed 39 specimens with known chromosome abnormalities. Eleven of these were in products of conception and are reported elsewhere. Of the remaining 28 specimens, 5 were double segmental imbalances, 3 were common trisomies, one was monosomy X, 4 were telomeric deletions, one was an interstitial duplication, and 14 had interstitial deletions. The microarray detected all of the abnormalities. Thus, the design of our array is ideal for identifying trisomies of any chromosome, known microdeletions and microduplications of the same loci, unbalanced derivative chromosomes, novel deletions/duplications of the pericentromeric regions, and for distinguishing consistent-sized deletions from larger deletions.

Searching for patients with the 22q11.2 duplication syndrome: Confirmation that some patients have phenotypic overlap with DiGeorge/Velocardiofacial Syndrome. A. Lamb¹, R. Kumar^{1,3}, J.E. Pellegrino², D. Chavez², T. Morris¹, P. Challinor¹, J.B. Ravnan¹. 1) Cytogenetics, Genzyme Genetics, Santa Fe, NM; 2) Inst Genetic Medicine, St Peter's Med Ctr, New Brunswick, NJ; 3) Univ Connecticut, Storrs, CT.

Microduplications of the 22q11.2 region had not been observed until Ensenauer et al (AJHG 73:1027-1040,2003) examined interphase cells by FISH with TUPLE1 in patients referred for DG/VCFS. They found 1.5% of unrelated patients had a duplication. To verify this finding and further characterize the phenotype associated with dup22q11.2, a prospective study was performed on 200 cases referred for DG/VCFS testing. FISH analysis on 100 interphase cells was performed. One patient with three copies of TUPLE1 was found (0.5%).

The patient was seen at 22 months and has Tetralogy of Fallot with aberrant subclavian artery, dysphagia, no overt cleft palate, moderate hearing loss, recurrent urinary tract infections with GU reflux, left cryptorchidism, abnormal EEG with seizures, hypotonia, global developmental delays, and hypoplasia of the corpus callosum. He had failure-to-thrive in infancy but currently at the 10%tile for weight and 25%tile for length. There is no documented immune deficiency. Dysmorphic features include: long narrow face, microcephaly, superior placement of eyebrows, bilateral ptosis with normal palpebral fissures, hypertelorism, bulbous nose, abnormal palmer creases. He does NOT have: bitemporal narrowing, brachycephaly, micrognathia, epicanthal folds, ear anomalies, long fingers and toes, or brachydactyly. His appearance is similar to patients in Ensenauer et al.

This study confirms that duplications of the 22q11.2 region can be found by examining interphase cells of patients referred for DG/VCFS. A subset of dup22q11.2 patients are being found due to overlap of features with DG/VCFS, but this does not find the majority of these patients as duplications should be as frequent as deletions. Focusing on the facial features along with LD/DD/MR without other features of DG/VCFS may uncover additional cases. Interphase cells should be examined for referrals for DG/VCFS.

Molecular cytogenetic investigations of multiplex autism families confirm 15q11-q13 duplications as a cause of autism. C. Lese Martin¹, J. Chung², Y. Ilkin², D.H. Geschwind³, D.H. Ledbetter¹. 1) Dept Human Genetics, Emory Univ Sch Med, Atlanta, GA; 2) Dept Human Genetics, Univ of Chicago, Chicago, IL; 3) Dept Neurology, UCLA Sch Med, Los Angeles, CA.

Autism is a neuropsychiatric disorder whose etiology is proposed to have a significant genetic component. Current data estimate that 2-6/1,000 children are affected with an autism spectrum disorder. A collaborative effort, the Autism Genetic Resource Exchange (AGRE), was previously initiated to collect and characterize multiplex families with autism for genetic studies. To cytogenetically characterize this valuable resource, we have used a combination of G-banding and Fluorescence *In Situ* Hybridization (FISH) analyses to examine lymphoblastoid cell lines from a subset of individuals in the AGRE collection. One affected individual per pedigree was analyzed. Of 146 individuals analyzed by G-banding analysis at a 400-550 band level, no abnormalities were identified. Genome-wide subtelomere FISH analysis was carried out to identify any submicroscopic rearrangements of these regions; no subtelomere rearrangements were identified in 128 individuals examined. Since 1-3% of individuals with autism have been shown to carry a duplication of the Prader-Willi/Angelman region on chromosome 15, targeted metaphase and interphase FISH analyses were performed using a clone for the *SNRPN* gene from this region. *SNRPN* FISH analysis was carried out on 148 individuals and two (1.4%) duplications were identified. Additional testing in these two families revealed that the other affected sibling in each family also carried the duplication, which was shown to be inherited from a phenotypically normal mother. Duplication of 15q11-q13 is the only known specific genetic cause of isolated and familial autism. These studies underscore the importance of clinical testing for the 15q duplication in autistic individuals, since the recurrence risk in such families is 50%. Furthermore, this study demonstrates the usefulness of cytogenetic analysis of individuals included in linkage studies, since subjects with chromosome rearrangements can be excluded from such analyses, thus increasing the study's power by reducing heterogeneity.

Development and application of a pericentromeric clone set for exploring genomic instability and architecture.

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The telomeric and pericentromeric regions of human chromosomes are of great biological and clinical importance. However, their complex, mosaic structures are rich in repetitive duplicated sequences, which have been recalcitrant to whole genome sequencing efforts. Targeted approaches are needed to elucidate their architecture, evolution and role in human disease. We previously reported a set of unique clones for each human telomere. Here we report the development of a set of pericentromeric clones corresponding to the most proximal unique DNA sequence for 43 chromosome arms, excluding the acrocentric p arms. A second clone, 500 kb distal to the first clone was chosen to initiate a molecular ruler for each region. These two clones were positioned in relation to the centromere and each other by interphase FISH. We assayed 112 clones, avoiding clones that showed greater than 10% segmental duplication using the UCSC genome browser. Of these 112 clones, 24% were excluded from analysis: 4 (3.5%) showed unique hybridization signals on the wrong chromosome; 22 (19%) had significant cross-hybridization to other loci by FISH and one showed a signal size polymorphism by FISH. These results demonstrate the necessity of targeted efforts in these regions as clone selection still remains problematic. Our final probe set consists of 85 unique FISH probes (only one unique clone was identified for Yp) that anchor the boundary between the unique and repetitive sequences for each pericentromeric region. We have utilized this probe set to examine 4 marker chromosomes identified during routine prenatal testing. Two marker chromosomes originated from chromosome 13 or 21 and two originate from 14 or 22. None of the 4 patients examined had positive FISH signals on their marker chromosomes suggesting that these markers do not contain euchromatin and may be compatible with a normal phenotype. Continued studies and extension of the molecular rulers will aid in the development of genotype/phenotype correlations that will result in better predictions of the clinical implications for marker chromosomes.

Identification of Chromosome Abnormalities by Array-CGH. *D.L. Pickering, M. Nelson, B.J. Dave, W.G. Sanger.*
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Cytogenetic and molecular cytogenetic studies play an important role in the study of mental deficiencies lacking specific clinical landmarks since they may be associated with small chromosome deletions and duplications. However, some chromosome abnormalities are submicroscopic and can only be resolved by specific FISH studies. With increasing genetic information, a growing number of critical chromosome regions need to be examined rendering FISH studies with multiple probes an expensive and time-consuming investigation. Array-CGH is a high-throughput molecular cytogenetic approach that allows for examination of the entire genome for gain or loss of several hundred specific chromosome regions in one assay. To test the reliability of array-CGH for the detection of chromosome abnormalities, we utilized the GenoSensor Genomic Array 300 (Abbott/Vysis, Downers Grove, IL) and examined nine cytogenetically abnormal cases and three cytogenetically normal cases with abnormal phenotypes. Array-CGH accurately identified the regions of deletion and duplication in the nine abnormal cases. Specifically, deletions of 1p36, 3ptel, 7ptel, 7q11.2, 8p22, 15q11.2-q13, 15q26.3, and 22q11.2, duplications of 5p15.2, 8q24.3, and 10p15 and a trisomy 18 were verified. Six of nine abnormal cases involved deletion and/or duplication of one or more subtelomeric regions. Additionally, in one case with der(15)t(8;15), precise breakpoint delineation within an 8Mb region of sub-band 15q26.1 to 15q26.3 was accomplished. The two cases with normal karyotypes were negative for chromosome deletions or duplications and another case with a balanced translocation was also negative for chromosomal gain or loss. Although this array was specific for the abnormalities tested, in eight of the 12 cases a false positive rate of 0.3-1.0% for gain or loss of random clones was noted. This emphasizes the need to confirm array-CGH results with FISH studies. When used in combination with cytogenetics and FISH, array-CGH is a useful tool for detection of subtelomeric and other subtle chromosome deletions and duplications and to help further define breakpoints in unbalanced karyotypes.

Fine mapping of 10qter deletions and duplications using QMPSF: importance of molecular rulers to distinguish deleterious from polymorphic rearrangements. *P. Saugier-Veber^{1,2}, V. Drouin-Garraud¹, A. Goldenberg¹, N. Le Meur^{1,3}, C. Carves¹, A. Rossi⁴, V. Leyer³, G. Joly-Hélas⁵, H. Moirot⁵, E. Bessenay¹, M. Tosi², T. Frebourg^{1, 2}* 1) Department of Genetics, Rouen University Hospital, 76031 Rouen, France; 2) Inserm U614, Faculty of Medicine, 22 Boulevard Gambetta, 76183 Rouen; 3) Department of Cytogenetics, Le Havre Hospital, 76083 Le Havre, France; 4) Department of Genetics, Etablissement Français du Sang, 76235 Bois-Guillaume, France; 5) Department of Cytogenetics, Rouen University Hospital, 76031 Rouen, France.

Constitutional subtelomeric imbalances account for approximately 6 % of idiopathic mental retardation (MR). We have developed a new simple molecular genetics assay based on QMPSF (Quantitative Multiplex PCR of Short Fluorescent Fragments) to detect and map subtelomeric rearrangements. Telomeric QMPSF is based on the simultaneous amplification under quantitative conditions of short fluorescent genomic fragments specific of each subtelomeric end. Telomeric QMPSF allowed us to identify one 10qter deletion and two 10qter duplications in MR patients and five 10qter duplications in normal controls. The 10qter deletion was detected in a 45 years old woman with MR, facial features and growth retardation compatible with previous 10qter deletion descriptions. The flexibility of the QMPSF method allowed the design of a new QMPSF, specific of the 10qter region, in order to estimate the size of each rearrangement. Seven amplicons spanning a 2 Mb region were integrated into this 10qter QMPSF. This analysis revealed that the 10qter deleterious deletion extended over more than 2 Mb. In contrast, the size of the polymorphic 10qter duplications could be estimated to less than 860 Kb. These comparative mappings of 10qter rearrangements using telomeric QMPSF show that deleterious and polymorphic rearrangements can both affect the 10qter region and that the polymorphic rearrangements may be distinguished from the deleterious ones by their size. Thus, because of its flexibility, telomeric QMPSF should facilitate the mapping of such subtelomeric rearrangements, with major implications in genetic counseling.

Deletion mapping in Xp21 for patients with complex glycerol kinase deficiency using Affymetrix GeneChip

mapping 10K array (SNP chip). *C.M. Stanczak¹, Z. Chen¹, M.K. Bedernik², Y-H. Zhang², S.F. Nelson¹, E.R.B. McCabe^{1,2,3}.* 1) Department of Human Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA; 2) Department of Pediatrics, David Geffen School of Medicine at UCLA and Mattel Children's Hospital at UCLA, Los Angeles, CA; 3) Molecular Biology Institute, UCLA, Los Angeles, CA.

Infantile, or complex glycerol kinase deficiency (cGKD), is a contiguous gene syndrome caused by a deletion of *GK*, along with its neighboring genes, *DMD* and/or *NROB1*. Patients with cGKD present with glyceroluria and hyperglycerolemia in association with Duchenne muscular dystrophy and/or adrenal hypoplasia congenita. The purpose of these investigations was to determine whether the Affymetrix GeneChip Mapping 10K Array (SNP chip) could be utilized to detect and map breakpoints in patients with cGKD. The Affymetrix SNP chip is a high-density oligonucleotide array that allows a standardized, parallel interrogation of 11,560 SNPs across the entire genome (except for the Y chromosome). Genomic DNA from 12 primary cell lines from patients with cGKD were analyzed on the Affymetrix platform. We identified deletions with the SNP chip in all 12 cGKD patients with a high degree of confidence. The no call rate and the average perfect-match minus mismatch intensity differed highly significantly (PE-5 for both comparisons) between the entire genome and the deleted regions. These data permitted us to specify minimum and maximum deletion intervals for each patient and these intervals compared well with independent results from PCR analysis and breakpoint sequencing. This study demonstrates the utility of the 10K mapping GeneChips for molecular cytogenetic analysis, beyond the SNP genotyping for which the arrays were initially designed. With 1% of live births (40,000 U.S. neonates annually) having cytogenetic disorders, we envision a significant need for such a platform for rapid, high-throughput, genomic analysis for molecular cytogenetics applications.

Analyses of structural abnormalities involving chromosome 4: Comparison between high-resolution multicolor banding technique and conventional G-banding. *K. Wakui^{1,2}, Y. Sakakibara¹, H. Ohashi³, K. Matsuda⁴, E. Hidaka⁴, T. Katsuyama⁴, Y. Fukushima^{1,2}.* 1) Division of Medical Genetics, Department of Health Science, Shinshu University School of Medicine, Matsumoto, Nagano, Japan; 2) Department of Clinical and Molecular Genetics, Shinshu University Hospital, Matsumoto, Nagano, Japan; 3) Division of Medical Genetics, Saitama Children's Medical Center, Iwatsuki, Saitama, Japan; 4) Department of Laboratory Medicine, Shinshu University Hospital, Matsumoto, Nagano, Japan.

High-resolution multicolor-banding (mBAND) technique was developed in 1999 to provide precise information on intra-chromosomal rearrangements (i.e., inversions, insertions and deletions) and exact break-point mapping (Chudoba et al. [1999], [2004]). We performed FISH analyses using chromosome 4-specific mBAND kit (Xcyte 4) by Isis mFISH/mBAND imaging system (MetaSystems) on the subjects with aberrations involving chromosome 4, identified by conventional G-banding. Xcyte 4 contains 6 region specific partial painting probes of chromosome 4, and each probe is labeled with 1 of 5 different fluorochromes or a combination of them, respectively. **Subject 1:** t(4;14;15)(q25;q31;q26.2), recurrent abortions; **Subject 2:** der(4)(4pter4q27::?:17q2317qter),der(17)(17pter17q21.3::4q284qter), MCA/MR; **Subject 3:** t(3;5;4)(q12.3;q31.3;q27), MCA/MR; **Subject 4:** t(4;6)(p16.1;q21),t(7;10)(p13;q22.3),inv(9)(p24.1;q12), MCA/MR; **Subject 5:** inv dup(4)(:p15.1p16.2::pterqter), MCA/MR. We reinvestigated the breakpoints of abnormal chromosome 4 in each subject, and compared and evaluated the results to conventional G-banding.

Phenotypic findings in patients with subtle terminal deletions detected by subtelomere FISH analysis. *G.D.*

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Subtelomere FISH analysis is an effective tool in the investigation of individuals with developmental delay (DD), idiopathic mental retardation, and various congenital anomalies. As these subtle or cryptic rearrangements are detected, it is important to distinguish between normal variants and pathogenetic rearrangements, and to delineate the phenotype associated with the latter. We have identified patients with subtelomeric deletions of 2q, 18p, and 11q. None of these was visible on GTG-banding analysis at 550-600 band resolution. Our patient with a de novo 2qter deletion was a 5 year old with autistic behavior, mild dysmorphism, atypical seizures, and was originally tested at age 3 to rule out Angelman syndrome. Patients with terminal 2q deletions have been reported. Common clinical findings include DD, frontal bossing, depressed nasal bridge, cardiac defects, and hypotonia. An association with autism or Albright hereditary osteodystrophy has also been reported. The patient with 18p deletion had failure to thrive at 8 months of age, dysmorphism, severe DD, cleft palate, and small stature. The biological parents were unavailable for testing. Deletion of 11qter was identified in an 11 month old with DD, dysmorphism, small mouth, and low-set ears, who was originally studied cytogenetically as a newborn to rule out trisomy 8 mosaicism. As increasingly subtle rearrangements are identified by subtelomere or DNA microarray analysis, discrimination of polymorphic variants and characterization of the phenotype represent challenges for the cytogeneticist and the clinician.

Characterization of a rare neocentric marker chromosome using chromosome microdissection. *J. Yu*^{1,2,3}, *Z. Qi*³, *K. Thompson*², *P. Modaff*⁴, *W. Wells*², *L. Meisner*^{1,2}, *R. Pauli*^{4,5}. 1) Dept of Pathology and Lab Medicine; 2) State Lab of Hygiene; 3) Waisman Center; 4) Dept of Medical Genetics; 5) Dept of Pediatrics, University of Wisconsin-Madison.

Chromosome microdissection is a unique technique by which any chromosome fragment within the cytogenetics resolution can be isolated and characterized. It is a powerful tool for clinical diagnosis. We present here an example of using this technique to study a low level mosaic neocentric marker chromosome. The patient was a 10 year old boy referred because of bilateral Perthes disease. In addition, he was noted to have mild developmental delays, attention deficit-hyperactivity, macrocephaly, and asymmetries of the hands and of the legs. He had linear and swirly arrays of irregular skin pigmentation and areas of focal skin atrophy, both distributed in a manner consistent with lines of Blashko. A normal male karyotype was detected in his blood cells. Because the skin pigmentation pattern strongly suggested possible mosaicism, we further analyzed skin biopsy samples. A marker chromosome was found in 4 skin cells. This marker was unstable, being lost in the passage cultures. Microdissection was used to further study this marker in very limited abnormal cells. A single copy of the marker was dissected in four contiguous sections, PCR amplified, labeled with multi-colors and hybridized to normal and patients metaphase cells. The hybridization results demonstrated that the marker was derived from the 3q26.2-qter region and lack of a centromere. Being able to be passed through multiple cell divisions, the marker most likely carried a neocentromere that has partial centromere function. The hybridization further demonstrated that 3q26.2-qter was present as an inverted duplication in the marker; this was confirmed by 3q telomere FISH. The findings greatly assisted the clinical assessment of the patient by providing a detailed and firm identification of the unstable marker that was most likely the cause of the abnormal phenotype. Our studies further demonstrated the necessity of using chromosome microdissection to completely analyze chromosome abnormalities that could not be confirmed by traditional methods.

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Clinical abnormalities in a girl with random X-inactivation and duplication of Xp11.1-p11.4. *D.S. Bonner, A.N. Meyer, J.E. Wiley, K.R. Schmidt.* Dept Pediatrics/ Genetics, E Carolina Univ, Brody Sch Med, Greenville, NC.

This report describes a 15 month-old female with abnormal clinical findings and *de novo* partial Xp duplication. She has mild dysmorphic features, a congenital heart defect, developmental delays (especially in the area of speech), and mild general muscular hypotonia. The cytogenetic analysis revealed a duplication of band p11.3 in the short arm of one of the X chromosomes (46,X,dup(X)(p11.2p11.4), with the normal and duplicated X chromosomes randomly inactivated. It has been postulated that cytogenetic or molecular replication studies alone cannot distinguish between phenotypically normal and abnormal females. In most females with structurally abnormal X chromosomes, the abnormal chromosome is inactivated and they are free from abnormal clinical findings. Some females with dup(X) chromosomes exhibit developmental defects because of random X-inactivation. A review of the literature did not reveal any similar case with this size duplication. This patient appears to be a first case of a female bearing this small duplication of Xp with random X-inactivation and may help to further define the location of genes important for development. There are several genes for mental retardation thought by linkage to lie within this band.

DEVELOPMENT AND VALIDATION OF CHROMOSOME MICROARRAY ANALYSIS (CMA) FOR CLINICAL DIAGNOSIS. *S. Cheung, W. Yu, C. Shaw, J. Li, Z. Ou, E. Brundage, S. Yatsenko, L. Cooper, S. Trilochan, P. Furman, A. Patel, P. Stankiewicz, W. Cai, J. Lupski, C. Chinault, A. Beaudet.* Department of Human and Molecular Genetics, Baylor College of Medicine, TX.

We have developed a second generation of BAC-based microarray at Baylor College of Medicine for clinical diagnosis of genetic disorders by comparative genomic hybridization (CGH). This array contains 380 FISH-verified clones that span genome regions implicated in over 40 known human genomic disorders. The array contains subtelomeric clones for the 41 clinically relevant human chromosome telomeric regions. The new array has multi-clone replication for each disease, with an average of 3-4 clones representing each deletion or duplication syndrome, and 4 or more clones for subtelomeric regions. The applicability of the chromosomal microarray in detecting copy number changes in the disease specific regions and subtelomeric regions was tested in a blinded fashion by hybridizing total genomic DNA from 25 patients to this array. A novel software package implemented in the R statistical programming language was developed for normalization, visualization, and inference. The overall results from the expanded array have been completely consistent with known cytogenetic and FISH abnormalities. Overall we find this to be a specific, sensitive and rapid approach for detecting chromosome imbalances. Two major advantages are the ability to test for a wide range of duplication and deletion syndromes without the need to rely on clinical suspicion to order specific FISH tests and the ability to detect duplications that might be undetected by metaphase FISH. The main limitation of this array is its inability to detect chromosome changes such as balanced translocations, inversions or low levels of mosaicism. However, a significant number of cryptic rearrangements, duplications or deletions may go undetected by typical chromosome analysis. Therefore, we conclude that the use of CMA together with standard cytogenetics can significantly improve diagnostic precision. We have now completed analysis of 55 prospective samples, and eight months experience offering the CMA test will be presented.

A de novo trisomy of 22pter-22q11.22 in a child with Ebstein's anomaly. R.A. Conte¹, L.A. Cannizzaro¹, V.R. Pulijaal¹, D.T. Walsh¹, D. Wei¹, M. Zohouri¹, T. Zhou¹, A.R. Deshikar¹, L. SanMarco¹, P.A. Levy², C. Walsh², K.H. Ramesh¹. 1) Pathology, Montefiore Medical Center/Albert Einstein Col. Med., Bronx, NY. 10461; 2) Pediatrics, Montefiore Medical Center, Bronx, NY. 10461.

A 7 year old Hispanic female with Ebstein's anomaly including tricuspid atresia, pulmonary atresia, mild mental retardation, asthma, decreased urine output and dysmorphic features:(slightly brachycephalic head, frontal bossing, hypoplastic midface, feet with mild pes planus, clubbing of fingers and hypertelorism) was referred for peripheral blood cytogenetic and FISH studies to rule out velocardiofacial syndrome. Neurological exam revealed mild hypotonia. Her dysmorphic features could not be categorized into any well-described syndrome. She was born at term bw:6lb 6oz to a 22-year-old G2 mother. Initially, FISH with N25 (D22S75)(22q11.2) and TUPLE1(22q11.2) loci probes, each with (ARSA) control (22q13.3), detected a third copy of N25 (D22S75) and TUPLE1 loci , on a deleted supernumerary chromosome 22 [SC22]. Additional FISH analysis using 22 wcp, 22q subtelomere (MS607) and centromere 22 (D22Z1) probes, in conjunction with GTG banded chromosomes, determined the karyotype to be: 47,XX,+del(22)(q11.22)[25].ish +del(22)(q11.22)(D22Z1+,wcp22+,N25+,TUPLE1+, ARSA-,MS607-)[120]. The parent's peripheral blood chromosomes were normal. Based on these findings the patient has a de novo chromosome abnormality of trisomy 22pter-22q11.22. It is believed that parental origin of SC22 does not have a strong influence on the progeny's phenotype since imprinting of chromosome 22 has not been reported. SC22s usually do not produce deleterious effects when their composition is noneuchromatic in nature. However, the amount of euchromatin that is trisomic, as a result of the SC22, plays a significant role in the phenotype. Additional molecular studies are warranted to further delineate this SC22 that may shed more light on the genotype/phenotype correlates involving Ebstein's anomaly in this child.

FISH Analysis for Prader-Willi Syndrome: Diagnostic Significance of Diminished Signal Intensity. *L. Dong, C. Wilczewski, L. Mak, B. White, J. Neidich, A. Anguiano.* Quest Diagnostics Nichols Institute, San Juan Capistrano, CA.

Prader-Willi syndrome (PWS) is a common dysmorphic syndrome characterized by hypotonia and feeding problems in infancy, followed by obesity, polyphagia, short stature, hypogonadism, behavioral disorders, and cognitive impairment. PWS is caused by molecular alterations of the SNRPN gene (15q11-q13) via microdeletion, duplication, uniparental disomy, and imprinting mutations. Approximately 70% of cases are detectable by FISH probes specific for the PWS SNRPN region. We present two cases: a 4-day-old female and a 3-month-old female, in which the clinical indication for FISH and cytogenetic analysis was hypotonia and feeding difficulties. Conventional cytogenetic studies for both patients were normal, with no visible deletion or duplication at 15q11-q13. A commercially available set of FISH probes (Vysis) that included a PWS-specific probe on chromosome 15 (SNRPN region [15q11-q13]) and two control probes (CEP 15 [15p11.2] and PML [15q22]) was used. In both cases, one of the chromosome 15 SNRPN regions consistently had a diminished signal. Follow-up studies were performed using proximal and distal probes adjacent to the SNRPN locus: D15S11 and D15S10, respectively. Both studies exhibited equal intensity on the target regions. Therefore, the FISH studies were reported as ish 15q12(SNRPN dim). Further investigation by methylation-specific PCR (MSPCR) of both cases showed an abnormal methylation pattern consistent with a clinical diagnosis of PWS. The probe manufacturer indicated that the 125Kb SNRPN probe preparation was sonicated into smaller 200-1000 bp fragments to allow for more efficient hybridization. This suggested that the observed diminished signal is likely due to a partial deletion within the SNRPN probe, leading to an alteration of the methylation pattern on 15q12. In conclusion, for SNRPN region FISH studies, when both signals are present, but one signal is consistently diminished, an additional test such as MSPCR is required to complete the evaluation.

Genomic array analyses of an unbalanced chromosome 18 translocation in a patient with psychosis. C.

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BACKGROUND: Array-based copy number analysis has recently emerged as an efficient means of mapping certain complex chromosomal abnormalities. Here we compare two such techniques in the evaluation of a 45 year-old female with dysmorphic features, mental retardation, psychosis, and an unbalanced chromosome 18p translocation.

METHODS: Patient genomic DNA was analyzed using both GeneChip 10K SNP Arrays (Affymetrix) and BAC-arrays provided by Roswell Park Cancer Center (RPCI) consisting of 6,000 probes with an average spacing of 0.5 Mb. Subtelomeric FISH with commercially available probes (Vysis) was used to confirm the origin of the translocated material. **RESULTS:** The patient's G-banded karyotype was read as 46, XX, der(18)t(18;?)(p12;?). Subtelomeric FISH confirmed the extra material was of chromosome 5 origin. SNP arrays mapped the duplication on chromosome 5p telomeric to position 23.630 Mb (July freeze; <http://genome.ucsc.edu>). BAC arrays placed the boundary at 25.320 Mb. The 18p deletion was mapped to an interval telomeric to either position 8.352 Mb (BAC array) or 8.437 Mb (SNP array). **CONCLUSIONS:** Array-based techniques are a rapid means of mapping difficult-to-characterize copy number changes, though at present they do not eliminate the need for traditional fine-mapping. Single hybridizations using either method defined a 25 Mb duplication on 5p and an 8.4 Mb 18p deletion. Interestingly, case reports and linkage analyses have implicated both chromosomal regions in psychosis. Further investigations of patients with such symptoms and cytogenetic abnormalities involving these intervals may help clarify the relative contributions of these loci to our patient's psychiatric presentation.

Constitutional t(8;22)(q24.1;q11.2): Prevalence and Clinical Significance. *S. Gupta¹, J. Joshi¹, R. Perrone¹, J. Gallo², S.L. Allen², P. Koduru¹.* 1) Dept. of Lab. Medicine, and; 2) Div. of Hematology & Oncology, North Shore Univ. Hospital, NY.

A reciprocal chromosome translocation (RCT) may be heritable or acquired; the latter are often associated with cancer. The most frequently reported heritable RCT is the t(11;22). t(8;22) has been reported in three different families (Godde-Salz et al 1982; Mark and Sigman 1999; Keung et al 2004). Although the positional relationship is not known, the 22q11.2 region has been a common site for rearrangements both in constitutional changes and in tumor related changes. We report a patient with t(8;22) ascertained because of thrombocytopenia. A 54 year male with a history of idiopathic thrombocytopenic purpura for 5 years developed worsening thrombocytopenia. His WBC was 12,000/uL, hgb was 15.2 g/dl, hct was 44.1, platelets were 36,000/ul, and differential count revealed 5% blasts. Flow cytometry demonstrated normal myeloid antigen pattern. Bone marrow aspirate and biopsy revealed increased megakaryocytes, erythroid hyperplasia, and absent iron stores. Chromosome analysis of a 24h cultured BM cells showed a t(8;22) (q24.1;q11.2); this was present in PHA stimulated PB cultures also. Therefore, the t(8;22) was a constitutional change in this patient. FISH study with cMYC, BCR, TUPLE1 and ARSA probes showed normal signal pattern; but BCR had moved to the 8q+ chromosome, cMYC had moved to the 22q- chromosome, TUPLE-1 was present on the 22q- chromosome, whereas the ARSA had moved to the 8q+ chromosomes. These findings suggest that the break at 22q11.2 occurred in-between TUPLE-1 and BCR genes, and the break at 8q24.1 occurred proximal to the cMYC locus. This is the second report of a patient with t(8;22) ascertained following the development of an autoimmune disease. A previous patient diagnosed with myasthenia gravis, leukocytosis and thrombocytosis has been found to have a t(8;22) (Keung et al 2004). Although certain constitutional changes such as del(13q), t(3;8), del(11p), have been reported to be associated with increased risk for cancer development, it is premature to consider whether the t(8;22) contributes to the development of autoimmune disease in carriers.

A second reported case of a female with dup (X)(q21.3-q24): case report and review of the literature. *K.C. Herman¹, P.N. Rao², R.E. Falk¹*. 1) Medical Genetics Institute, Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) Department of Pathology and Laboratory Medicine, UCLA Medical Center, Los Angeles, CA.

We report a 22-month-old Hispanic female with global developmental delay, hypotonia, joint laxity, poor feeding, failure to thrive, and minor dysmorphic features. Cytogenetic analysis performed shortly after her birth revealed an abnormal female karyotype consistent with segmental duplication of the long arm of the X chromosome from band q21.3 to q24. The duplication was confirmed by whole chromosome painting of the X chromosome. Cytologically, the morphology of the X chromosomes suggested random X-inactivation, but specific X-inactivation studies have not been completed.

A review of the literature found one other case of interstitial duplication of Xq with similar breakpoints, reported by Carozzo et al (*Am J Med Genet.* 72:329-34, 1997). This female infant presented with developmental delay, hypotonia, intrauterine growth restriction and subsequent short stature. However, the reported dysmorphic features were quite different than those seen in our case. This child was found to have random X-inactivation, an unusual finding as most reported cases of females with dup(X) have skewed X-inactivation. An MRI study obtained at 20 months revealed findings suggestive of hypomyelination. The gene involved in Pelizaeus-Merzbacher disease, PLP, is located at Xq22, within the region duplicated in this child. FISH testing confirmed duplication of the PLP gene.

The evaluation of our patient is on-going. However, her neurologic features are similar to the previously reported child. Also, considering the cytologic appearance of random X-inactivation, this may represent a second case of unexpected random X-inactivation in a female with dup(Xq). Possible explanations for this phenomenon have been hypothesized, including impaired cell selection mechanisms, a lethal mutation on the normal X chromosome, and interference with the mechanism leading to the preferential inactivation of the abnormal X chromosome.

Array-based comparative genomic hybridization facilitates identification of breakpoints of der(1)t(1;18) in a child presenting with mental retardation and clinical features of autism. *P.A. Lennon¹, C.A. Bacino¹, M.A. Curtis², C. Lim², Z. Ou¹, M.L. Cooper¹, A. Patel¹, S.W. Cheung¹.* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Pediatrics, University of Arkansas for Medical Sciences College of Medicine, Little Rock, AR.

Duplications involving 18q rearrangements produce clinical phenotypes ranging from severe, as seen in trisomy 18, to milder, as seen with a smaller duplication involving only 18q2118qter. To this date, no duplication of 18q has been reported involving only the distal 18q2318qter. We report dup(18)(q23qter) as well del(1)(p36.3) which were detected via subtelomere fluorescent in situ hybridization (FISH) and array-based comparative genomic hybridization (array-CGH) during the cytogenetic analysis of a 7 1/2 year old female with a der(1)t(1;18)(p36.3;q23). Thus, this patient has a combination of abnormalities which include duplication 18q phenotypic anomalies as well as features often seen in del(1)(p36) syndrome. At birth, a large anterior fontanelle and mild clinodactyly of both 5th fingers were noted. On clinical exam at age 7 1/2 she had dysmorphic features which included a pointed chin, a broad nose with a flat nasal bridge, mild hypertelorism and a midface hypoplasia. These findings are typical for the clinically recognizable del(1)(p36) phenotype. She also had an open mouth and widely-spaced teeth. She had a history of chronic ear infections superimposed to a moderate bilateral sensorineural hearing loss. Dextrocardia was previously diagnosed at birth. Global developmental delay was evident and she displays features seen in the autistic spectrum disorders. Molecular characterization of this novel der(1)t(1;18)(p36.3;q23) involves determining the precise chromosomal breakpoints, which may yield insight into this compounded phenotype as well as into subtelomere healing. These breakpoints have been elucidated using array-CGH and FISH, and will be presented.

A Referral Center for Prenatal FISH Study in Taiwan First Year Experience. *C.C. Lin¹, Y.C. Li², L.J. Hsieh¹, C.P. Chen³, T.M. Ko⁴, C.C. Tzeng⁵, M.L. Yang⁶, J.S. Fang⁷, M.S. Li⁴, L.S. Tzeng⁸, Y.J. Pan⁹, S.K. Lin⁶, F.J. Tsai¹.* 1) Dept Medical Research, China Medical Univ Hosp, Taichung, Taiwan; 2) Dept Life Sci, Chung Shan Med Univ, Taichung, Taiwan; 3) Mackay Memorial Hosp, Taipei, Taiwan; 4) Kos Obs/Gyn Clinic and M.S. Li Obs/Gyn Taipei/Taichung, Taiwan; 5) Chi-Mei Med Ctr, Tainan, Taiwan; 6) Vet General Hosp Taipei/Taichung Taiwan; 7) Tai-An Hosp, Taichung, Taiwan; 8) Natl Taiwan Univ Hosp, Taipei, Taiwan; 9) Lin Shin Hosp, Taichung, Taiwan.

Several large series of prenatal interphase FISH have been reported. Much lesser population data were available for prenatal diagnosis using FISH-based full karyotyping analysis. Since 2003, a referral center for FISH-based study has established to offer prenatal FISH analysis for clinical cytogenetic laboratories in Taiwan. We reported here the experience for the first year of such service. There were 10 cytogenetics laboratories using the service with a total of 19 samples or fixed cells submitted for FISH-based analysis. This represented 0.26% of total number of prenatal cytogenetic cases (7,143) performed in those laboratories. All referral cases were requested for full karyotype FISH study including 9 (47%) cases of de novo marker chromosomes, 3 (16%) cases of suspected microdeletion syndromes (2 cases for DiGeorge syndrome and 1 case for Williams syndrome) and 7 (37%) cases of undetermined structural aberrations. The chromosome origins of all marker chromosomes were identified by SKY except for 1 case which was identified by FISH with Y-specific probe DYZ3. 3 of the markers (33%) were derived from the chromosome 22. No deletion was detected in all 3 cases suspected of having microdeletion syndromes. Among structural aberrations identified, 4 cases involved extra chromosomal material of various origin, 1 case of isochromosome, and 2 cases of cryptic reciprocal translocations. The identifications of chromosome origin and structural rearrangements may be important for genetic counseling to couples undergoing prenatal testing. The study was supported by grants from The Bureau of Health Promotion, Taiwan (BH92-GC03-1) and from NSC, Taiwan (NSC 91-2320-B-040-037).

Two novel cases of duplications with cryptic gain or loss of the IgH gene. *P. Papenhausen¹, J. Tepperberg¹, I. Gadi¹, L. Wisniewski¹, P. Jayakar², U. Lichter-Konecki³, S. Sparks³.* 1) Dept Cytogenetics, Labcorp of America, Res Triangle Pk, NC; 2) Miami Children's Hospital, Clinical Genetics, Miami, FL; 3) Children's National Medical Cntr, Div of Genetics, Washington, DC.

A 22 mo old female was referred for cytogenetic evaluation secondary to failure to thrive (ht&wt at the 3rd percentile) and mild speech delay. A mosaic peripheral blood karyotype revealed 35% of the cells had an added segment at 9p23 and 65% were normal. Ancillary FISH studies showed tandem 9p subtelomere positivity representing a predictable terminal 9p duplication, but with an intervening G-band negative "spacer" inconsistent with distal 9p G-banding. Sequential WCP-FISH revealed an insertion from chromosome 14. Both the light band color and the nature of the IgH gene suggested that it may be a candidate region for the insert and gene specific FISH confirmed the compound mosaic partial trisomies for 14q32.2-q32.3 and 9p23-pter.

The second case was a 7mo old male referred for cytogenetics and subtelomere FISH due to a VSD/ASD, dysgenesis of the corpus callosum, hypotonia and DD. The pregnancy was complicated by oligohydramnios, IUGR and premature birth at 33wks. G-banded karyotypes showed a subtle "swelling" of the 14q32 band which was deleted of the subtelomere in the FISH analysis. No subtelomere of any chromosome was noted in that analysis consistent with many distal inverted duplications in the literature. Additional gene specific FISH for the IgH gene showed that it was part of the terminal deleted segment and 14 WCP showed that the added segment was from chr 14 and consistent with a duplication. The duplicated segment was then presumed to be 14q32.1-q32.2. The transposability of the IgH gene may be associated with the etiology of the structural alterations in both of these cases, resulting in the distal duplications. Clinical comparisons with similar cases will be presented. Competence of CGH chip analysis in revealing the overall imbalance in these cases will be reported.

Deletion of 22q11.2 associated with a jumping translocation. *D.L. Persons¹, X.Y. Wang¹, D. Collins², T. Arthur¹, S. Hagemeister¹, J. Ragona¹, B. Davoren¹, T. Troyer¹, R.N. Schimke².* 1) Department of Pathology and Laboratory Medicine; 2) Department of Internal Medicine, University of Kansas Medical Center, Kansas City, KS.

Jumping translocations are rare chromosomal rearrangements that are defined as translocations involving the same portion of a donor chromosome and various recipient chromosomes in different cell lines of a single person. Only a limited number of cases (approximately 24) involving constitutional jumping translocations have been reported in the literature. Four of the reported cases involved chromosome 22; three of which had a clinical history consistent with deletion of 22q11.2. Fluorescence in situ hybridization (FISH) was not performed to verify a deletion at the molecular level in these cases. We report a six-year-old male who presented with velopharyngeal incompetency. He was without the classical facial features of velocardiofacial syndrome (VCFS). Parathyroid hormone and calcium levels were normal and an echocardiogram was unremarkable. Chromosomal analysis revealed three different cell lines, each containing an unbalanced translocation involving the long arm of chromosome 22 from q11.2 to qter. In the majority of cells (87%), chromosome 22 was translocated to 11p15.5; two minor populations contained translocations of 22q11.2 to 6q27 (10%) and 4p16.3 (3%). FISH using a DNA probe specific for the DiGeorge/VCFS critical region (TUPLE1) at 22q11.2 confirmed that 100% of 35 metaphases examined had the deletion. In addition, subtelomere probes for 11p and 6q were present in all metaphases examined and were not rearranged. Parental cytogenetic studies and FISH studies for deletion 22q11.2 were normal. This is the first case where deletion of 22q11.2 associated with a jumping translocation was verified using FISH.

Postnatal detection of nonmosaic tetrasomy 18p in an infant with multiple congenital abnormalities, characterized by G-banding and FISH. A. Piurek¹, J. Meck¹, E. Zafer¹, N. Scribanu², D. Boles¹. 1) ObGyn, Georgetown University Hospital, Washington, DC; 2) Pediatrics, Georgetown University Medical Center, Washington, DC.

Tetrasomy 18p was identified postnatally in a newborn infant by high-resolution karyotype analysis and confirmed by FISH. The pregnancy was uncomplicated; prenatal tests were normal, except for a low baseline fetal heart rate and maternal hyperemesis gravidarum. Multiple congenital anomalies and dysmorphic features were observed at birth including: bilateral talipes equinovarus; slanted palpebral fissures; retromicrognathia; adducted thumbs; capillary hemangiomas on the forehead and occiput; small, low set and rotated ears; prominent nose; short neck; bilateral transverse palmar creases; hypertonicity; grade II/VI heart murmur; and both atrial and ventricular septal defects. The proband's mother and maternal uncle also had VSDs at birth. Heart valve defects, feeding difficulties, hypertonicity, short neck, craniofacial deformities, low set ears and hand/foot deformities have been reported previously by others as well (Takeda et al., 1989, Pinto et al., 1998). G-band analysis revealed a small supernumerary metacentric chromosome that appeared to consist of 18p material in all cells. FISH was performed using probes specific to the telomeres and centromere of chromosome 18. All metaphases examined had 18p telomere probe signals on each end of the supernumerary chromosome. The centromere also appeared to be enlarged indicating duplication of the centromeric region. Proximal 18q11.2 is also believed to be duplicated based on the G-band appearance of the supernumerary chromosome. The karyotype was 47,XY,+idic(18)(q11.2).ish idic (18q11.2)(18ptelx2, D18Z1x2). Parental bloods were karyotypically normal. A two month follow up was significant for several ear infections and severe bilateral hearing loss. The patient has neck and right arm contractures, similar to a report by Pinto et al. (1998). The feeding problems have resolved; weight is now at the 50th percentile. Subsequent pregnancies in the parents will be monitored with prenatal genetic testing in the unlikely event of gonadal mosaicism.

Monte Carlo Simulations in Clinical Cytogenetics: Modeling the Detection of Abnormal Cell Subpopulations. *K. Shera*¹, *G. Bonnet*¹, *C.A. Shera*². 1) Cytogenetics Studio, Santa Fe, NM; 2) Harvard Medical School, Boston, MA.

The American College of Medical Genetics has proposed quality standards and guidelines for clinical cytogenetic analysis (ACMG, 2003). For specimens of peripheral blood or bone marrow, the ACMG recommends that a minimum of 20 metaphase cells be evaluated, and in many US clinical cytogenetics laboratories, evaluation of 20 cells constitutes a routine clinical cytogenetic analysis. When test sensitivity is 100%, a 20-cell analysis is sufficient to exclude mosaicism or an abnormal clonal subpopulation of approximately 15% with 95% confidence (Hook, 1977). A larger sample is required to: (1) rule out lower levels of mosaicism or clonal abnormality, or (2) maintain power when test sensitivity is less than 100%, as in cancer or molecular cytogenetic analysis. How large a sample is large enough? In statistical terms, a cytogenetic analysis represents a simple random sample of size n , drawn from the total population of cells collected from the patient under study. We used Monte Carlo simulation, a powerful stochastic method, to model the random sampling of cell populations harboring different proportions of chromosomally abnormal cells, while varying test sensitivity and sample size. For a test of a defined sensitivity, we determined the sample size required to detect a given abnormal cell subpopulation, assuming a variety of sampling distributions. When test sensitivity was 85% (as in some FISH analyses), exclusion of a clonal abnormality of 20% required analysis of 300 cells; a 200-cell analysis failed to detect the clone between 9-16% of the time, depending on the variance in test sensitivity. Our study highlights the fundamental role of sample size in quality clinical cytogenetic analysis. As a practical aid to the clinical cytogenetics lab, we present a series of Monte Carlo detection curves, simple tools for estimating the minimum sample size required to reliably detect a given abnormal cell subpopulation when using a test of a known sensitivity, or conversely, the confidence level attained in excluding that subpopulation when a defined number of cells is analyzed. The detection curves are applicable to both conventional and molecular cytogenetic analysis.

Newborn with diaphragmatic hernia and single cell with marker chromosome: think Pallister-Killian syndrome.

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The infant was born at 30 weeks gestation to a 33-year-old female. During the pregnancy, an ultrasound at 24 weeks identified a diaphragmatic hernia. The stomach was in the thoracic cavity medial to the heart. Polyhydramnios, bilateral short femurs and a left renal cyst were also present. The patient received genetic counseling and declined amniocentesis. A fetal echocardiogram at 25 weeks was within normal limits. The mother had a history of poorly controlled type 1 diabetes. Family history was significant for a maternal uncle who died at 3 years of age with a congenital heart defect, and two paternal half-nephews with congenital deafness. The mother was hospitalized at 29 weeks with preterm labor. Nine days later the fetal membranes ruptured and a Cesarean section was performed due to fetal heart distress. The birth weight of the male infant was 1240 g, with APGAR scores of 4/5/6 at 1, 5, and 10 minute, respectively. A peripheral blood sample was drawn for chromosome analysis. The newborn died on the second day of life due to respiratory distress syndrome. The karyotype was normal with 1 of 20 cells containing a small metacentric marker chromosome, thought to be an isochromosome 12p. The marker was confirmed as being an isochromosome 12p using an alpha-satellite FISH probe for chromosome 12 in interphase cells. An autopsy was not performed on the patient. Paraffin slides on the umbilical cord and placenta were obtained to examine other tissue. The percentage of interphase cells with the isochromosome 12p was 43% in peripheral blood with more than twice as many signals in non-stimulated cells (68%) versus stimulated cells (24%). In umbilical cord cells, 35% contained 3 signals, similar to 30% in the placenta. To our knowledge, this is the third reported case that has examined paraffin slides to determine or confirm mosaicism in multiple tissues for Pallister-Killian syndrome.

Automated detection of metaphase chromosomes in FISH and routine cytogenetics. *P. Yanala^{1,2}, T. Lu², F. El-Ghussein^{1,2}, C. Zhao², D. Medhi^{1,2}, Y-P. Wang², J. Knopp², J.H.M. Knoll¹, P.K. Rogan^{1,2}.* 1) Children's Mercy Hospital, Schools of Medicine and; 2) Computer Science and Engineering, University of Missouri-Kansas City.

We present an integrated, automated microscopy system that has been designed to assist the cytogeneticist in rapidly selecting the optimal metaphase spreads for routine cytogenetic or FISH analyses. After the boundaries of the coverslip are localized, slides are raster-scanned and either DAPI-stained or GTG-banded metaphase cells are distinguished from other objects at low magnification. Autofocusing at both low and high magnification maximizes contrast across features in the image based on Brenner's Sharpness Measure. Procedures for determining the optimal focal position could be expedited at low power, based on the fact that focal planes of adjacent fields of view are nearly identical. The metaphase detection algorithm has a specificity of 98% and sensitivity of 54%. The centroids of these metaphase cells are relocated and recentered at high power magnification (100X objective), and, for FISH, DAPI and probe images are captured using automated exposure controls that are customized for each channel. Metaphase images are ranked using a content-based classification system trained on features extracted from chromosome spreads. The ranking procedure differentiates metaphase cells with limited chromosomal overlap from incomplete or poorly spread metaphases. This algorithm has an average precision of 88% with an 80% recall rate relative to ranking by an experienced cytogeneticist. Multichannel images are uploaded to a modified version of the web-based Open Microscopy Environment, where they are merged and indexed for subsequent retrieval or recapture. The integrated expert system (a) substantially increases the throughput: allowing many more probes to be analyzed in the same time frame; (b) mitigates fluorescent bleaching due to prolonged light exposure; (c) efficiently manages large volumes of cytogenetic data; and (d) potentially reduces errors due to eye strain, thereby improving identification of chromosomal abnormalities.

Recurrent sites for new centromere seeding. *M. Ventura*¹, *S. Weigl*¹, *L. Carbone*¹, *M.F. Cardone*¹, *D. Misceo*¹, *M. Teti*¹, *P. D'Addabbo*^{1,2}, *A. Wandall*³, *E. Bjork*⁴, *P.J. de Jong*⁵, *X. She*⁶, *E.E. Eichler*⁶, *N. Archidiacono*¹, *M. Rocchi*¹. 1) Dept Dapeg Sez Genetica, Univ Di Bari, Bari, Italy; 2) Center for Research into Molecular Genetics Fondazione CARISBO, Institute of Histology and General Embryology, University of Bologna, Bologna, Italy; 3) Dept. of Medical Genetics, Panum Institute, Copenhagen, Denmark; 4) Department of Molecular Medicine, Karolinska Institutet, Stockholm Sweden; 5) Children's Hospital Oakland Research Institute, Oakland, CA; 6) Department of Genetics, Case Western Reserve University School of Medicine, Cleveland, OH.

Using comparative FISH and genomics, we have studied and compared the evolution of chromosome 3 in primates and two human neocentromere cases on the long arm of this chromosome. Our results show that one of human neocentromere cases maps to the same 3q26 chromosomal region where a new centromere emerged in a common ancestor of the Old World monkeys approximately 25-40 million years ago. Similarly, the locus where a new centromere was seeded in great apes ancestor was orthologous to the site where a new centromere emerged in New World monkeys ancestor. These data suggest the recurrent use of longstanding latent centromeres and that there is an inherent potential of these regions to form centromeres. The second human neocentromere case (3q24) revealed unprecedented features. The neocentromere emergence was not accompanied by any chromosomal rearrangement that usually trigger these events. Instead, it involved the functional inactivation of the normal centromere, and was present in an otherwise phenotypically normal individual who transmitted this unusual chromosome to the next generation. We propose that the formation of neocentromeres in humans and the emergence of new centromeres during the course of evolution share a common mechanism.

Hidden 45, X/47, XXX/47, XX, del(Y)(p?)/46, XX mosaicism associated with True Hermaphroditism (TH). *K. Nieto¹, S. Kofman-Alfaro¹, R. Alvarez¹, Y.R. Pena², I. Palma¹, L. Erana², L.M. Dorantes², G. Queipo¹.* 1) Dept. Genetics, Hospital General de Mexico, Mexico City, Mexico; 2) Intersex Clinic. Hospital Infantil de Mexico-Federico Gomez, Mexico City, Mexico.

True hermaphroditism (TH) is an unusual form of sex reversal, characterized by unequivocal testicular and ovarian elements in the same individual. Approximately 60% TH have a 46, XX karyotype, 33% are mosaics with a second cell line containing a Y chromosome, while the remaining 7% are 46, XY. The Sex determining gene *SRY*, is present in only 10% of true hermaphrodites with a 46, XX karyotype, therefore in the remaining 90%, mutations at unknown X-linked or autosomal sex determining loci have been proposed as factors responsible for testicular development. TH presents considerable genetic heterogeneity with several molecular anomalies leading to the dual gonadal development in a single subject. In 1998, gonadal *SRY* mosaicism was proposed in 3 sporadic 46, XX TH *SRY*- negative in blood leukocytes but positive in gonadal tissue and in 2002 the presence of hidden mosaicism for Y-derived sequences was confirmed as an alternative mechanism to explain TH etiology in some cases. Here we report a new TH case in which a hidden mosaicism for the Y and X chromosome was detected by PCR and FISH in peripheral blood and gonadal tissue, supporting the fact that hidden mosaicism is associated with TH and that molecular analysis of gonadal tissue should be done in all 46, XX cases.

A sub-microscopic duplication of Xq27.1 including the *SOX3* gene is associated with hypopituitarism. M.

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Recent studies have implicated *SOX3* in the etiology of X-linked hypopituitarism. Sox3 belongs to the B1 subfamily of Sox transcription factors and marks neuroepithelial progenitor and stem cells from the earliest stages. Expansion of a polyalanine tract within *SOX3* has been implicated in X-linked mental retardation associated with isolated GH deficiency (IGHD), and interstitial duplications encompassing *SOX3* at Xq26.1-q27.3 have been associated with variable GHD and hypopituitarism. To date, the smallest duplication identified spans 9Mb.

We screened a cohort of males with hypopituitarism and identified a family in which 2 siblings (variable hypopituitarism with absent infundibulum and anterior pituitary hypoplasia) have been shown to harbour a sub-microscopic duplication of 690kb on chromosome Xq27.1. Increased dosage of *SOX3* was confirmed by interphase FISH using a BAC probe containing *SOX3*. The chromosome rearrangement in this family is small and was not identified by standard chromosome analysis, and we have shown that the duplication is tandem in nature. The extent of the duplication has been characterised by interphase FISH in this family using further probes from the region and the duplication breakpoints have been identified.

The duplicated region in this family included just the *SOX3* gene and two novel uncharacterised genes. This, to our knowledge, is the smallest duplication described to date. Since *SOX3* has already been associated with hypopituitarism we suggest that increased dosage of this gene is the primary cause of the pituitary abnormalities in this family.

Evidence Of Preferential Meiotic Segregation In Two Balanced Translocations. *J. Cuzzi*¹, *C.G. Petersen*², *A.C. Laus*¹, *A.L. Mauri*², *R.L.R. Baruffi*², *J.G. Franco Jr*², *L. Martelli*¹. 1) Genetics Department, University of Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil; 2) Human Reproduction Centre, FMSJ, Ribeirao Preto, Sao Paulo, Brazil.

Chromosome translocations are associated with aneuploid gametes, which may be responsible for embryos with unbalanced karyotypes, first trimester abortions, fetal losses or liveborns with a chromosomal syndrome. Preimplantation genetic diagnosis (PGD) is an early form of prenatal diagnosis, which makes possible to detect hereditary genetic diseases in human embryos. In this study, we present the PGD results in two young couples, with normal phenotype, presenting recurrent abortions. In the first one (couple A), the spouse was the carrier of robertsonian translocation involving chromosomes 13 and 14, with karyotype 45,XX,der(13;14)(q10;q10) and in the second (couple B), she was the carrier of reciprocal balanced translocation involving chromosomes 7 and 18, with karyotype 46,XX,t(7;18)(q31;q22). Our objective was to establish a molecular cytogenetic diagnosis, to indicate the transfer of euploid embryos. In Couple A, seven embryos were biopsied and 6 nuclei were submitted to fluorescent in situ hybridization (FISH) technique, using 14q telomeric and LSI 13q14 probes. All nuclei showed 2 signals (compatible with normal or balanced karyotype) corresponding to chromosome 14, but presented aneuploidy of chromosome 13, three of them being monosomic and two trisomic. In Couple B, five embryos were biopsied and 5 blastomeres were obtained and submitted to FISH technique, using 7q and 18q telomeric probes. All nuclei showed 2 signals related to chromosome 18, but presented aneuploidy of chromosome 7, three of them being trisomic and one monosomic and the diagnosis was inconclusive in one blastomere. Therefore, the transfer of the embryos was not indicated in both cases. Our results in both patients make evident a mechanism of preferential meiotic segregation where all oocytes were abnormal for one of the involved chromosomes in the translocations. PGD was able not only to select the embryos, but also to define the risk for the offspring, fetal losses and the birth of an affected child.

Molecular cytogenetics analysis for diferencial diagnosis of lissencephaly. *A.C. Laus¹, A.C. Santos², S.A. Santos¹, J.F. Cuzzi¹, E.S. Ramos¹, L.R. Martelli¹.* 1) Genetics, University of São Paulo, Ribeirão Preto, São Paulo, Brazil; 2) Radiology, University of São Paulo, Ribeirão Preto, São Paulo, Brazil.

Classical or type I lissencephaly (LIS) is a malformation characterized by smooth cerebral surface (cortical agyria-pachygyria) originated by incomplete neuronal migration during embryonic development. LIS can be described in two forms: Miller-Dieker Syndrome (MDS) and Isolated Lissencephaly Sequence (ILS). MDS consists of lissencephaly, characteristic facial abnormalities, sometimes associated to other birth defects, and almost 100% of the patients present deletion of LIS1 gene, located to 17p13.3. The ILS main finding is the cortical malformation, without other abnormalities. This type can be associated with alterations in LIS1 or DCX gene, located to Xq22.3-q23, which encodes proteins associated with neuronal movement. The aim of this study was to perform molecular cytogenetic analysis of the short arm of chromosome 17 in ten patients with lissencephaly to determine the diferencial diagnosis and to provide adequate genetic counseling to their families. We have performed routine cytogenetic and molecular cytogenetic analysis in patients presenting lissencephaly confirmed by magnetic resonance image. The cytogenetic studies included GTG high resolution banding; for the FISH protocols we have used specific probes located to 13.3 and 11.2 regions and telomeric region of the short arm of chromosome 17. The results did not show any numerical or structural chromosomal abnormalities and any deletion in 17p13.3 region nor in the tel17p region. The protocol was useful to exclude the diagnosis of Miller-Dieker syndrome in all patients and to formulate the diagnosis of X-linked lissencephaly in one family, changing the recurrence risks for the families. According to the literature lissencephaly results from complex genetic and biochemical mechanisms. In our study we have analyzed just the 17p13.3 and telomeric regions. Therefore, we suggested that other mechanisms, as intragenic mutation in LIS1 gene undetected by FISH, alterations in DCX gene, alterations in PAFAH1B2 or PAFAH1B3 subunits or even some environmental factors could explain the cortical malformation.

Molecular Cytogenetic Anchors for the Zebrafish Genome. *C. Lee*^{1,2}, *H. Stern*^{1,2}, *C.D. Belair*^{2,3}, *Y. Zhou*^{2,3}, *B.H. Paw*^{2,3}, *L.I. Zon*^{2,3}, *A. Smith*¹. 1) Department of Pathology, Brigham & Woman's Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) Children's Hospital, Boston, MA.

The zebrafish, *Danio rerio*, is an important model organism for studying vertebrate development, cell biology and cancer. The zebrafish genome is organized into 25 pairs of chromosomes that are difficult to unequivocally identify solely on the basis of classical cytogenetic methods that rely on chromosome size, morphology and dark-and-light banding patterns. Systematic mapping of large-insert clones to metaphase chromosome spreads from wildtype zebrafish will provide molecular cytogenetic anchors for the zebrafish genome, which in turn could be integrated with the well-developed linkage maps. We have now mapped a total of 74 BAC clones, containing genomic DNA inserts of an average of 170 kb. Each clone has been end-sequence verified prior to FISH-mapping. Flanking z-markers serve as reference points for relative positioning of these clones on the various available linkage maps. With the exception of the chromosome corresponding to linkage group 4, three clones were identified for each chromosome: one near each chromosome end (telomere) and one near the primary constriction (centromere). The apparent presence of a substantial amount of heterochromatin on the long arm of the linkage group 4 chromosome has precluded identification of a specific near-telomeric probe for this chromosome region. These DNA probes are extremely useful for assessing chromosome translocations, ploidy and genomic instability in mutants and tumor material. These molecular cytogenetic anchors also refine current zebrafish linkage maps, providing a framework that underlies the correct assembly of the DNA sequence of the zebrafish genome.

Program Nr: 1004 from the 2004 ASHG Annual Meeting

Numerical chromosomal anomalies of the tissues involved in neural tube defects (NTD). *D.R. Lucon, D.P. Cavalcanti.* Perinatal Genetics Program, Dept. Medical Genetics, UNICAMP, Campinas, Sao Paulo, Brazil.

Chromosomal anomalies have been described in a number of children displaying neural tube defects. Interphasic FISH was suggested to be an appropriated tool analyzing criptomosaicism in malformed babies. In a recent paper it was reported chromosomal variation in neurons of both, developing and adults mammalian nervous system. The purpose of this study was to investigate tissue involved in neural tube defects (TINTD) applying interphasic FISH technique. We report the results of the investigation from 13 cases of children presenting neural tube defects (NTD). In the studied samples, cytogenetical and karyotype analysis were performed from peripheral lymphocytes. Also, analysis of the TINTD by conventional karyotype and interphasic FISH were realized. Standard procedures for cytogenetics analyses were employed. At least 15 metaphases from lymphocytes and 15 from the TINTD for each case were studied by GTG banding. Interphasic FISH analysis was performed using the multiprobe system (Cytocell), and from each case, at least 300 nucleous were accounted. With the purpose of defining the confidence limit, a control sample was taken from peripheral lymphocytes of 10 clinically normal newborns. The results showed four mosaicisms: three tetraploid/diploid cases and one monosomy 6. The monosomy 6 and one case of $4n/2n$ were detected only by the interphasic FISH of the TINTD. It was also detected a probable pseudomosaicism represented by a monosomy 8. The conclusions are that 7.7 % chromosomal numeric anomalies were found in lymphocytes from peripheral blood, 15,4% in TINTD by conventional cytogenetics, and 25% in TINTD by interphasic FISH. These results suggest that numerical anomalies could be very common in tissues involved in neural tube defects. Moreover, these results suggest that further studies with neural tissues in humans are fundamental to clarify the relationship between chromosomal numerical anomalies and nervous tissues. (Supported by FAPESP, 98/16006-6).

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Breakpoint analysis of a balanced reciprocal t(3p;10p)translocation in a Bardet-Biedl syndrome patient. *H.L. May-Simera, P.J. Scambler, P.L. Beales.* Molecular Medicine Unit, Institute of Child Health, London, United Kingdom.

Bardet-Biedl syndrome (BBS) is a heterogeneous disorder with primary features that include age-related retinal dystrophy, obesity, polydactyly, renal dysplasia, reproductive tract anomalies and cognitive impairment. Eight loci have been found to be causative for the disease (*BBS1-8*), for which seven have been cloned. Recent evidence suggests that aspects of the BBS phenotype may result from defective ciliogenesis/basal body function. We identified a female Iranian BBS patient with a paternally inherited balanced reciprocal translocation t(3;10)(p13;p15.3) in whom we have excluded mutations in known BBS genes. Given the coincidental G-banding karyotype localisation of the breakpoint in 3p13 with the published linkage interval for *BBS3* (3p13-12), we performed FISH analysis on EBV transformed PBL to determine if breakpoints are associated with gene disruption. The 3p breakpoint mapped outside the locus for *BBS3*, but localised within an incomplete region of the genome sequence. Although we can exclude this region from *BBS3* we cannot yet rule out the possibility of an additional disease-related gene at 3p14. Likewise, the breakpoint on 10p potentially disrupts two candidates, one of which is directly involved with microtubule assembly and intracellular transport. We report results of our translocation mapping approach in an attempt to identify a novel BBS gene.

Analysis by fluorescence in situ hybridization (FISH) of the relation between gonosomal aneuploidy and the results of assisted reproduction in men with severe oligospermia. *M. MEHDI, M. BENCHAIIB, B. SMATTI, J.F. GUERIN.* Laboratory of Biology of reproduction, Faculty of Medicine, University Claude BERNARD, Lyon, France.

OBJECTIVE: The aim of this study was to analyze the frequency of sex chromosome aneuploidy in human spermatozoa of severe oligozoospermic men undergoing intracytoplasmic sperm injection (ICSI), in order to evaluate the impact of these chromosomal anomalies on the results of the ICSI. **MATERIALS AND METHODS:** Fluorescent in situ hybridization (FISH) with direct label fluorescence DNA probes specific for chromosome X, Y and 18, was performed on decondensed spermatozoa from fresh ejaculates of 12 patients with severe oligozoospermia undergoing ICSI. A total of 500 spermatozoa were analyzed per chromosome per probe per donor. **RESULTS:** The rate of gonosomal aneuploidy was significantly increased in the patients compared with the case in the normal donor (respectively 3,5 and 0,8%). The sex-chromosomal anomalies due to the I meiosis (XY) are less important than the anomalies due to the II meiosis (XX or XY), but the difference was not statistically significant. There was a negative correlation between the rate of aneuploidy and the percentage of spermatozoa with normal morphology ($r = -0,71$; $p < 0,05$). The correlation was negative between the percentage of gonosomal aneuploidy and the rate of fertilization ($r = -0,7$; $p < 0,001$). In conclusion, our results suggest an increased rate of gonosomal aneuploidy in the patients with oligozoospermia compared with the normal population. This aneuploidy, although it decreases the rate of fertilization, doesn't seem to affect the rate of segmentation, nor the embryonic quality.

Program Nr: 1007 from the 2004 ASHG Annual Meeting

Sperm aneuploidy in the male partners of the couples with Bad Obstetric History. *Z.M. Patel, A. Meka, H.M. Gawde, S.R. Menon.* NIRRH/GRC, Indian Council Medical Research, Jehangir Merwanji Street, Parel Mumbai, India.

Approximately 15% of all conceptions end in spontaneous abortions in the first trimester and 60% of it bear chromosomal abnormalities. Of the various vital factors involved, sperm aneuploidies may be one. Thus, the objective of this research was to investigate the relationship between sperm aneuploidy and spontaneous abortions (simultaneously looking into other factors). The study also aims at looking into correlation between sperm morphology, count and aneuploidies. 20 semen samples (200000 cells) from male partners of the couples with 2 or more abortions, were analyzed for aneuploidies for chromosomes 13, 21, 18, X, and Y using multicolor LSI (13,21) and CEP (18, X&Y) FISH probes. FISH analysis showed marked deviation from normal only in 5 patients of the 20 analyzed. The mean frequency of total disomic sperm (X-X, X-Y, Y-Y, 13-13,21-21) was 14.65 per 10^4 cells (range 1- 190), frequency of total diploid sperm was 10.1 per 10^4 cells (range 7-175) and frequency of total nullisomic sperm was 10.75 per 10^4 cells (range 1-75). Details of sperm count and percentage of abnormal forms were also obtained wherever possible and they were analyzed for correlation. The analysis reveals no correlation between aneuploidies and sperm morphological abnormalities as well as between aneuploidies and sperm count. Our study suggests that sperm morphology and count in themselves are not sufficient to rule out the role of sperms in spontaneous abortions.

Developmental Genome Anatomy Project (DGAP): In Search of Genes Critical for Human Development. *F. Quintero-Rivera*^{1,4}, *G.A. Bruns*^{2,4}, *D.J. Donovan*³, *R. Eisenman*², *H.L. Ferguson*³, *D.J. Harris*^{2,4}, *A.W. Higgins*^{3,4}, *A.H. Ligon*^{3,4}, *H.G. Kim*^{1,4}, *K.M. Kocher*⁴, *W. Lu*^{3,4}, *R.L. Maas*^{3,4}, *S.D. Moore*^{3,4}, *N.T. Leach*^{3,4}, *R. Peters*³, *B.J. Quade*^{3,4}, *I. Saadi*^{3,4}, *R.E. Williamson*⁴, *C.C. Morton*^{3,4}, *J.F. Gusella*^{1,4}. 1) Massachusetts General Hospital, Charlestown, MA; 2) Children's Hospital, Boston, MA; 3) Brigham & Women's Hospital, Boston, MA; 4) Harvard Medical School, Boston, MA.

DGAP (<http://dgap.harvard.edu>) represents an integrated approach to finding genes important in human development by studying individuals who have apparently balanced chromosomal rearrangements and congenital anomalies. Our progress to date includes ascertainment of 160 cases, 71 FISH-mapped breakpoints, and localization of 48 breakpoints from 33 cases within single BAC clones. Candidate genes have been identified in 23 cases and breakpoints cloned in 7 cases. Of 13 breakpoints analyzed in detail, 9 reside within the intron of a gene, none is within an exon, one occurs within a 3' UTR, and 3 are present in putative non-genic regions with the breakpoint in the other derivative chromosome disrupting a known gene. Microdeletions, insertions and duplications involving a few nucleotides have been detected in 6 cases and presently are interpreted not to be of pathological significance. Evaluation of possible conserved non-genic sequences in these introns is being studied in addition to assessing of candidate genes. Mutational analyses of patients with phenotypes similar to a DGAP case are being performed in 8 cases. Knock-out mouse models of 6 candidate genes from 5 DGAP cases are being created; chimeric mice have been obtained for all 6 genes and breeding for germline transmission is underway. As the number of mapped cases increases, the DGAP database will provide a resource to predict clinical severity of *de novo* balanced chromosomal rearrangements diagnosed pre- and post-natally. Because it is currently not possible to make such clinical prognoses, this database will be a valuable contribution in the improvement of genetic counseling and subsequent decision-making. DGAP investigators thank the many geneticists worldwide who make this project possible.

22q11 rearrangements in different patients groups. *M. Kriek¹, K. Szuhai², S. White¹, S. Kant¹, J. Knijnenburg², J. Dauwerse¹, C. Rosenberg², J. den Dunnen¹, M. Breuning¹.* 1) Center for Human and Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands; 2) Molecular Cell Biology, Leiden, Netherlands.

The 22q11 region contains 4 highly homologous sequences, known as low copy repeat sequences (LCR). Despite the difference in size and organisation of the repeats, the overall sequence identity is 97-98 %. This homology predisposes for unequal recombination between the different LCRs. Different genomic disorders caused by rearrangements within 22q11, are characterized by an enormous variation in clinical features with no obvious relation between the size of the genomic imbalance and the severity of the clinical characteristics. To investigate 22q11 alterations in different patients groups, we have screened 96 adult patients diagnosed with Tetralogy of Fallot (TOF) as well as 220 mild to severe developmental delayed (DD) patients with a normal karyotype using Multiplex Amplifiable Probe Hybridisation (MAPH) and Multiplex Ligation-dependent Probe Amplification (MLPA). Seven deletions of 22q11.2 were diagnosed among the TOF patients (7.3%), a figure similar to that reported by other studies. The screening of DD patients resulted in the detection of 2 alterations; in one case a duplication of 22q11.2, and in the other a more complex chromosome rearrangement. By MAPH analysis, we demonstrated a deletion on 22q11.1 and a duplication on 22q11.2. Additional arrayCGH analysis revealed a second deletion on chromosome band 22q12 in the same patient. Since Fluorescent In Situ Hybridisation (FISH) experiments had already excluded the patients presenting a typical 22q deletion phenotype from our samples, the number of alterations detected is rather high (~1%). The limited number of described duplications on 22q11.2 is probably due to a combination of phenotypic diversity and difficulty of diagnoses by FISH analysis on metaphases. MLPA represents a better technical alternative for diagnosing copy number changes in 22q11, as it simultaneously detect deletions and duplications. Moreover, in contrast to FISH experiments, MLPA can target small rearrangements and does not depend on dividing cells for analysis.

Variation in meiotic recombination frequencies among human males. *F. Sun*^{1,2}, *K. Trpkov*³, *A. Rademaker*⁴, *E. Ko*², *R.H. Martin*^{1,2}. 1) Dept Medical Genetics, Alberta Children's Hosp, Calgary, AB, Canada; 2) Department of Medical Genetics, University of Calgary, Calgary, Canada T2N 4N1; 3) Department of Pathology, Rockyview Hospital, Calgary, Canada T2V 1P9;; 4) Cancer Center, Biometry Section, Northwestern University Medical School, Chicago, USA, 60611-4402.

Meiotic recombination (crossing over) is essential for the segregation of homologous chromosomes and formation of normal haploid gametes. Little is known about patterns of meiotic recombination in human germ cells or the mechanisms that control these patterns. The aim of this study was to determine the extent of variation in the frequency of recombination in human males and to uncover any variables contributing to this variation. Newly-developed immunofluorescence techniques, based on the detection of MLH1 (a DNA mismatch repair protein) foci on synaptonemal complexes (SCs) at prophase of meiosis, were used to examine recombination in human spermatocytes. The mean number of MLH1 foci per cell in 11 donors was 48.0 with range from 21 to 65. Remarkable variation in the recombination frequency was noted among 11 normal individuals: the mean frequencies of chromosomal recombination foci ranged from a low of 42.5 to a high of 55.0 exchanges. Donor age did not contribute to this variation. There was no correlation between this variation and the frequency of gaps (discontinuities) or splits (unpaired chromosome regions) in the SCs. The mean percentage of cells with gaps was 35% (range 20-58%) and with splits was 7% (range 0-37%). In our observations of more than 20,000 autosomal SCs, the proportion of bivalents without a recombination focus was 0.3%. Thus, achiasmate chromosomes appear to be rare in human male meiosis.

Interstitial Deletions of Chromosome 6q: Genotype-Phenotype Correlation of Three Patients Utilizing Array Comparative Genomic Hybridization. *O. Klein¹, P. Cotter¹, A. Zanko¹, M. Gilats¹, C. Epstein¹, F. Conte¹, D.*

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Interstitial deletions of chromosome 6q are relatively rare, with approximately 50 cases reported. Phenotypic variation is due to differences in size and location of the segmental aneuploidy. Here, we report three new patients with interstitial deletions of chromosome 6q characterized by array CGH. Patient 1 had a conventional karyotype 46,XY,del(6)(q15q16.2) and had craniofacial dysmorphism including brachycephaly, downslanting palpebral fissures and low-set, dysplastic ears. He also had retinitis pigmentosa and strabismus. Patient 2 had a karyotype 46,XY,del(6)(q16.2q21). He had IUGR, and craniofacial dysmorphism included brachycephaly, hypotelorism with downslanting fissures and dysplastic ears. The patient also had a visual abnormality, minor hand and foot anomalies, truncal obesity and delayed myelination on brain MRI. Patient 3, whose conventional karyotype was 46,XY,del(6)(q15q16.2), had low-set ears, a visual abnormality, minor hand and foot anomalies, truncal obesity and diabetes insipidus. All patients were developmentally delayed. To define the molecular breakpoints and deletion size, array CGH analysis was performed. Deleted regions were as follows: Patients 1 and 2 had minimal deletions of 6 Mb involving 6q16.2q21 and 8.8 Mb involving 6q16.2q21, respectively. Patient 3 had the largest deletion of 11.3 Mb spanning 6q15q21. All three had developmental, ear and eye anomalies, suggesting genes for brain and craniofacial development in 6q16.2q21, the deleted region common to the patients. Furthermore, gene(s) for discordant features, such as diabetes insipidus, may reside at 6q15, the monosomic region unique to Patient 3. In two of three cases, the molecular breakpoints differed from those indicated by conventional karyotyping, demonstrating the enhanced resolution of array CGH. The improved characterization of segmental aneuploidy by array CGH may impact the prediction of a patient's phenotype and prognosis, as well as provide finer mapping of candidate genes for specific malformations.

Phenotypic features in a boy with trisomy of 16q22-qter due to paternal Y;16 translocation. A. Talarski^{1,2}, F. Tsien², E. Morava³, M. Marble^{1,2}. 1) Genetics, LSU Health Science Center, New Orleans, LA; 2) Children's Hospital of New Orleans, New Orleans, LA; 3) UMC Nijmegen, The Netherlands.

The incidence of Y;autosome translocation in newborns is approximately 1/10,000, with the long arm of chromosome Y most frequently involved. Most patients with either unbalanced or apparently balanced Y-autosome translocations are infertile, usually due to disruption of one of the DAZ genes in the azoospermia factor (AZF) region in the euchromatic area of the Y chromosome long arm. We report an 8-year old patient with partial trisomy of the long arm of chromosome 16. High resolution chromosome analysis revealed an unbalanced Y;16 translocation [46,X,der(Y)t(Y;16)(q12;q22)pat], which was confirmed by fluorescence *in situ* hybridization (FISH) using whole chromosome paint probes for chromosomes Y and 16. The patient was noted to have craniofacial anomalies including a prominent forehead, thin upper lip, and smooth philtrum. He also had growth deficiency and mental retardation. The father, a balanced Y;16 translocation carrier, has apparently normal fertility since the Y chromosome breakpoint is in the heterochromatic region distal to the DAZ genes. He has no dysmorphic features. There have only been a few published reports of trisomy for 16q22-qter. In these previous cases, phenotype/cytogenetic correlations have been difficult to assess due to the overlapping effects of the other involved chromosome. In contrast, since the translocation breakpoint in our patient involves Y heterochromatin, it is likely that the patient's phenotype is reflective of true 16q22-qter trisomy. Analysis of additional cases of trisomy 16q will help to further delineate the trisomy 16q phenotype.

IS THE PRADER-WILLI SYNDROME PHENOTYPE RELATED TO MOLECULAR MECHANISMS? A STUDY OF 75 PATIENTS. *M.C. Varela¹, F. Kok², N. Setian³, C.A. Kim⁴, C.P. Koiffmann¹*. 1) Human Genome Study Center, Department of Biology, Bioscience Institute, University of Sao Paulo, Sao Paulo, SP, Brazil. email: mcvarela@ib.usp.br; 2) Child Neurology Service, Dept of Neurology, Univ of Sao Paulo School of Medicine; 3) Endocrinology Unit, Childrens Institute, Univ of Sao Paulo School of Medicine; 4) Childrens Institute, Univ of Sao Paulo School of Medicine.

Prader-Willi syndrome (PWS) can result from either a 15q11-q13 paternal deletion, maternal uniparental disomy (UPD), or imprinting mutations. We describe here the phenotypic variability detected in 51 patients with different classes of deletions and 24 patients with UPD. Diagnosis was made by methylation pattern analysis of exon 1 of the SNRPN-SNURF gene and by microsatellite profiling of loci within and outside the 15q11-q13 region. Although no statistically significant differences could be demonstrated between the two main classes of PWS deletion patients, it was observed that Class I (BP1-BP3) patients acquired speech later (4 years 4 months) than Class II (BP2-BP3) patients (3 years 3 months). Comparing the clinical pictures of our patients with UPD with those with deletions, we found that: UPD children presented lower birth length and started walking earlier, at age 2 on the average, whereas in deletion patients the average was 2 years and 5 months; deletion patients presented a much higher incidence of seizures (45%) than UPD patients (7.14%); the mean maternal age in the UPD group was higher than in the deletion group. No statistically significant differences could be demonstrated between the deletion and the UPD group with respect to any of the major features of PWS (neonatal hypotonia, poor sucking and failure to thrive in the postnatal period, delayed psychomotor development and facial features). In conclusion, our study did not detect significant phenotypic differences among Class I and Class II PWS deletion patients, but it demonstrated that seizures were six times more common in patients with a deletion than in those with UPD. This finding might be accounted for by haploinsufficiency of genes related with seizures (as GABRB3) in the deleted region. Supported by: FAPESP, CEPID, CNPq.

Recurrent Trisomy 21: Four Cases in Three Generations. *J.L. Gair¹, R. Rupp¹, L. Arbour¹, R. Jiang¹, H. Bruyere², W.P. Robinson¹.* 1) Dept. of Medical Genetics, University of British Columbia, Vancouver, BC Canada; 2) Dept. of Pathology, University of British Columbia, Vancouver, BC, Canada.

While gonadal mosaicism can lead to recurrence of trisomy 21 for a single couple, recurrence of free trisomy 21 in multiple members of a single pedigree has rarely been reported. We hereby present an unusual pedigree with four cases of Down syndrome (DS) born to four separate women related through three generations of one family. The mothers were aged 18, 21, 29 and ~30 at the time of the births. G-banded karyotypes in two of the mothers of DS individuals were normal and karyotypes of all of the DS individuals showed free trisomy 21. Microsatellites spanning chromosome 21 were typed in this family to determine if any regions were shared in common among the mothers of the DS children. The centromeric and telomeric regions of chromosome 21 could be excluded from being shared, and of those tested, only the markers D21S215 and D21S258 (21q11.2) and D21S1440 (21q22.3) were potentially shared. Two members of the pedigree including one DS mother carried supernumerary alleles at markers 2503J9TG, D21S369 and D21S215, which span the region from 21p - 21q11.1. The level of meiotic recombination on chromosome 21 was unusually high in this family as well. FISH using a centromeric probe for chromosome 21 did not reveal a novel location which rules out a cryptic translocation. We hypothesize that there is a complex rearrangement involving the centromeric region that disrupts the function of the centromere and leads to nondisjunction of chromosome 21 in this family. This is currently being tested with multicolour FISH using BAC probes on either side of the centromere.

Paternally derived 15q12-q13 duplication associated with Autism Spectrum Disorder. *M. Smith, M. Hanouni, M.A. Spence, J.J. Gargus, M.A. Lerner.* Dept. Pediatrics, Univ. California Irvine, Irvine, CA.

Studies by Cook et al., Huang et al., (1997) concluded that 15q duplications that arise on a maternal chromosome and contain the Prader Willi Angelman (PWA) syndrome critical region, lead to manifestations of autism spectrum disorder (ASD). There are a few reports of subjects with paternally derived duplications of chromosome 15q that include the PWA region who manifest developmental problems (Mohandas et al 1999) or ASD (Roberts et al., 2002). We describe studies on a female subject who had hypotonia, developmental and speech delay: walked at 15 months, did not use simple phrases until the age of 3 years. She had difficulty learning to read. Multisensory reading instruction and individualized learning assistance was provided from age 7- 15 years. At 12 years she met criteria for autism in 3 domains: Social interaction, Communication, Repetitive behaviors and interests. Cumulative CARS Score: 30.5 (Mildly Autistic). Assessment at 17 years revealed short stature, minimal dysmorphism and motor co-ordination problems. Cognitive assessment revealed superior verbal and normal performance IQ, normal reading skills and dyscalculia. Cytogenetic studies revealed an interstitial duplication of 15q12-q13. Plasma levels of lactate, pyruvate and ammonia were normal. Skin fibroblast activities of mitochondrial respiratory complexes 2-4 were normal. In these parameters she differs from patients with ASD and 15q inverted duplication that we reported (Filipek et al., 2003). Analysis with 8 different Bac clones showed that the interstitial duplication includes the PWA region between breakpoints BP2 and BP3. Analysis of polymorphic micro- satellite markers revealed that proband has three alleles at each of the following loci: D15S122, GABRB3, D15S1002 and GABRA5. At each of these loci the patient inherited two alleles from her father and one allele from her mother. We conclude that paternally derived duplications of the PWA region may lead to manifestations consistent with autism spectrum disorders. Furthermore the molecular lesion potentially sub-localizes critical regions involved in the metabolic and developmental components of the 15q duplication ASD syndromes.

In vitro assessment of the genotoxicity of benzo-a-pyrene using the trypan blue exclusion test, the sister chromatid exchanges (SCE) and the electron microscopy in situ end-labeling (EM-ISEL) tests on human blood lymphocytes.

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Benzo-a-pyrene (BaP) is a polyaromatic hydrocarbon (PAH) compound, listed by the International Agency for Research on Cancer (IARC) as a probable human carcinogen. It is used as a model for the carcinogenicity of the 20 PAHs listed as priorities by the IARC. PAHs are produced during the incomplete combustion of organic matters, where hundreds of different compounds are emitted simultaneously, making it hard to assess the effects of the exposition to carcinogenic PAHs in the workplace or in the general environment. In our study, human cultured lymphocytes were exposed in vitro to different concentrations of BaP dissolved in DMSO, 0,1g/ml, 1g/ml and 10g/ml, for 24 hours. Cells obtained from 5 different donors, selected following the same criteria, were harvested 24 hours after the end of the exposition and assessed for viability, SCE and EM-ISEL. The viability test showed no cytotoxic effects, attesting that our doses are suitable for genotoxic studies. A significant increase in the mean number of SCEs per cell was found in the cells exposed to 10g/ml of BaP. A significant increase in the amount of DNA single-strand breaks (clastogenic effect) was present in the cells exposed to a lower dose (1g/ml of BaP). Our data show that, in the same experimental conditions, a genotoxic effect of BaP is detected at a dose lower with EM-ISEL test than the minimal genotoxic dose found with the SCE test. Apart from that, we detect a clastogenic effect of BaP at lower doses and shorter exposition times, when compared to similar studies reported in the literature. Pursuing this study on a larger number of participants will allow us to determine if a variation in the genotoxic response following exposure to benzo-a-pyrene exists between individuals.

Preimplantation genetic diagnosis (PGD) for chromosome translocations: Strategies for probe selection. *J.-C. Wang, A. Hajianpour, R. Habibian.* Genzyme Genetics, Pasadena, CA.

Couples with balanced translocations are at risk for spontaneous abortions or abnormal liveborns as a result of unbalanced meiotic segregants. Application of PGD can decrease the risk of these adverse outcomes by selectively transferring only normal or balanced embryos. Various strategies have been employed using combinations of FISH probes. These include chromosome paint probes, centromeric probes, subtelomeric probes, other unique sequence probes, and probes spanning or flanking translocation breakpoints. The latter probes, while most specific, require custom development and are impractical for most labs. A complete set of chromosome-specific subtelomeric probes is now available commercially. Unbalanced translocations can be detected using two subtelomeric probes labeled with different fluorophores, making PGD feasible in most cytogenetics labs. However, two-probe hybridization can not detect all types of unbalanced segregants. With 2:2 and 3:1 segregations, there are 14 different products. Of the 12 unbalanced products, some can result in viable fetuses. It is imperative that these potentially viable products be detected by PGD. Intuitively, the probes to be used are the subtelomeric probes located at the two translocated segments. This will detect all but the two adjacent-2 segregants. Although most adjacent-2 products are exceedingly unbalanced, fetuses can occasionally survive to term when translocation segments are extremely large. Furthermore, virtually all unbalanced liveborns of carriers of the most common reciprocal translocation in humans, t(11;22)(q23.3;q11.2), have tertiary trisomy due to 3:1 segregation. Therefore, probe selection needs to be individualized to avoid transfer of undetectable unbalanced embryos that can result in viable fetuses. It will be shown that probes can be selected that will detect both adjacent-1 and -2 segregants but specifically miss two of the eight 3:1 segregants, or detect all but adjacent-1 segregants. Alternative probe sets can also be used depending on the availability of differentially labeled probes, as long as the two undetectable segregants are not compatible with long term fetal survival.

Pregnancy outcome following prenatal diagnosis of 46,XX,dup(9)(q22.3q32): first case report. *M. Do¹, H. Lin², M. Thangavelu¹, K. Chan³, D.B. Rogers¹.* 1) Genzyme Genetics, Orange, CA; 2) Harbor-UCLA Medical Center, Torrance, CA; 3) Magella Medical Group, Torrance, CA.

We report a case of an 11-month old girl with a partial duplication of chromosome 9q diagnosed prenatally. The mother was seen for genetic counseling because of an abnormal expanded AFP screening test (positive for Down syndrome). Ultrasound at 18 weeks was unremarkable and she underwent an uneventful amniocentesis. The fetal karyotype revealed extra material on the long arm of one chromosome 9 homolog. Further cytogenetic analyses revealed the karyotype to be *de novo* 46,XX,dup(9)(q22.3q32). Fluorescence in situ hybridization confirmed the karyotype. Following further genetic counseling, the mother elected to continue the pregnancy. Pregnancy complications included oligohydramnios and reduced movement at 39 weeks. Labor was induced, and a viable SGA female infant was delivered. Remarkable features at birth included a 31-week head circumference, facial hypertelorism, large set ears and possible cardiac malposition. Postnatal peripheral blood chromosome analysis confirmed the prenatal diagnosis. Following minor feeding problems the infant went home after 3 days. At four months and eleven months, the infant demonstrated poor postnatal growth, persistently falling under the 5th percentile for both weight and height. She was found to have microcephaly, low frontal hairline, bulging forehead, arched eyebrows, upslanted palpebral fissures, hypertelorism, upper lip over lower lip/probable retromicrognathia, fifth finger clinodactyly, and a cardiac murmur. Evaluation of gross and fine motor skills, cognitive and language at eleven months indicated that the infant was developing within the normal range. This child manifests a number of features documented in previous reports of partial trisomy 9q, including facial dysmorphism, microcephaly and pre- and postnatal growth delay. However, this is the first report of normal development in what appears to be a duplication of a unique segment of the long arm of chromosome 9.

Second Trimester Trisomy 22: Cytogenetics and Ultrasound Findings. *S.A. Ebrahim^{1,3}, M. Treadwell^{2,3}, A.N. Mohamed^{1,3}.* 1) Department of Pathology, Cytogenetics Laboratory; 2) Department of Obstetrics and Gynecology, Hutzel Hospital; 3) Wayne State University, Detroit, Michigan.

Trisomy 22 usually presents in cases of early spontaneous abortions and is a rare finding in the second and third trimester fetuses. A 29 year-old gravida 4, para 3, VIP 0, female was referred for genetic counseling and amniocentesis due to anomalies detected on ultrasound scan. Ultrasound revealed a growth-retarded fetus (15 weeks by ultrasound and 20 4/7 weeks by last menstrual period). Diffuse skin edema was noted. The upper and lower extremities remained in a flexed position throughout the exam. There appeared to be an underdevelopment of the right side of the heart. FISH on uncultured amniocytes showed no evidence of numerical abnormality for chromosome 13, 18, 21, X, and Y. Subsequent chromosome analysis of cultured amniocytes revealed a 47,XX,+22[20]. The trisomy 22 colonies were distributed in five independently established cultures. The patient was counseled regarding these findings, and opted to continue the pregnancy. Subsequent ultrasound two weeks later revealed new onset non-immune hydrops with ascites now present. The previously noted persistent flexion of the extremities was still apparent. The cardiac defect appeared most consistent with an unbalanced endocardial cushion defect with a large ventricular septal defect. At 20 weeks gestation the pregnancy is still ongoing.

MOLECULAR CHARACTERIZATION OF TWO CONSTITUTIONAL RINGS DERIVED FROM ONE CHROMOSOME 22 DETECTED DURING PRENATAL DIAGNOSIS. *M. Gadjji¹, K. Krabchi¹, M. Ferland¹, S. Cote², M. Perigny³, P. Langis⁴, R. Drouin¹.* 1) Dept of Med Genet & Dept of Pediatrics, CHUS, Univ. de Sherbrooke, Sherbrooke, Qc, Canada; 2) Cytogenet Lab, CHUL, CHUQ, Qc, Canada; 3)) Sce de Path, Hp. St-Francois d'Assise, CHUQ, Laval Univ., Qc, Canada; 4) Sce de Radiologie, Hp. St-Francois d'Assise, CHUQ, Laval Univ , Qc, Canada.

Introduction: Several cases of ring chromosome 22 have been described in the literature. We present the first case of two constitutional rings derived from one chromosome 22. **Objective:** Cytogenetic and molecular genetic characterization of two rings chromosome 22 identified during prenatal diagnosis. **Methods:** A 39 year-old, gravida 4, para 2, abortion 1 woman had amniocentesis performed at 16 3/7 weeks of gestation for AMA. Conventional and molecular cytogenetic studies of the fetal and parental cells were performed to allow the diagnosis. Microsatellites analyses were performed to elucidate the parental origin and the genetic pathogenesis. **Results:** The fetus had two rings chromosome 22 with three breakpoints: one located at the centromere (22q10), another one at the band 22p11.2 and the third one at the band 22q13.31. The distal parts of the chromosome were lost. Then, it resulted two rings: one constituted by joining the end of band 22p11.2 and a portion of the centromere leading to a small ring; the other by joining the second part of the centromere and the end of band 22q13.31 leading to a larger ring. The male fetus presents the following karyotype: 47,XY,r(22)(p10p11.2),+r(22)(q10q13.31). The parental karyotypes were: 46,XX,16qh+ (mother); and 46,XY,inv(9)(p11q12)(father). The probands chromosome aberration occurred de novo from the maternal chromosome. The breakpoint is localized between the microsatellite markers D22S1170 (22q13.31) and D22S922 (22q13.32). At the autopsy, the fetus showed slight clinical features. The number of fetal nucleated blood cells detected in peripheral maternal circulation, showing positive signals for Y chromosome and DiGeorge/VCF probes with absence of ARSA control probe signal was 10 cells per ml. **Conclusion:** Despite the haploinsufficiency of many active genes, the fetus showed very slight congenital malformations.

Further characterization of a 46,X,abn(X)/45,X karyotype detected prenatally. *P. Koduru¹, S.D. Batish³, S. Dittmar¹, C. Moore², L. Mehta², S. Gupta¹.* 1) North Shore Univ. Hospital, Manhasset, NY; 2) Schneider Children's Hospital at NSUH; 3) Ahena Diagnostics, Inc., Worcester, MA.

Precise diagnosis and characterization of genomic imbalance in patients with sex chromosome abnormalities may help in the evaluation of phenotype, genetic counseling and clinical follow up. We report here use of cytogenetics, FISH and molecular studies in characterizing a psu dic(X) in a newborn girl. Amniocentesis was performed on a 36 y.o., G1P1 woman for reasons of maternal age. FISH on AF cells detected one signal for the X chromosome in 94.3% cells and two signals in 3.7% cells. Chromosome analysis showed mosaicism for two cell lines, mos 46,X,dup(X)(?q24q26)[3]/45,X[42]. The pregnancy was continued and at 37 weeks of gestation a non-dysmorphic baby girl was born. Renal sonogram was normal, she had a small atrial septal defect. Blood chromosome analysis from the newborn confirmed the prenatal findings. FISH study with probes for X- paint, centromere and subtelomere regions was performed. The WCP probe painted the whole abn(X), suggesting that the structural alteration involved only the X. X-centromere probe detected one signal in 62.58% nuclei and three signals in 37.42% nuclei. The Xp subtelomeric probe hybridized to the p-arm of the normal X and to both ends of the abn(X). The Xq subtelomeric probe did not show signal on the abn(X), but hybridized to the q-arm of the normal X. These findings suggest that the abn(X) lost a part of the distal region of Xq, but had duplication of the short arm. Therefore, the karyotype of this abnormal line was 46,X,abn(X).ish psu dic(X) (pter->q22.1::q22.1->pter)(tel Xp+ +,DXZ1+ +,tel Xq-). Chromosome analysis on mother was normal. DNA studies with polymorphic markers at Xq27.3(FMR1) and Xq12(AR) showed heterozygosity at both loci suggesting the presence a of 46,XX cell line not detected by cytogenetic methods. This implies that the abnormality was post-zygotic in origin. Despite the mosaicism for XX/dup(Xp)/X cell lines, the majority of cells are 45,X. Therefore, the infant is predicted to have some features of Turner syndrome, and appropriate follow-up is planned.

Mosaic marker i(21q) resulting in tetrasomy 21 identified through an integrated prenatal screen (IPS) positive for Down Syndrome. *E. Mak-Tam*¹, *M. Care*¹, *D. Chitayat*², *J. Furnival*¹, *P. Griffin*¹, *A. Summers*¹, *P. Wyatt*¹. 1) Genetics Program, North York General Hospital, Toronto, Ontario, Canada; 2) The Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, Ontario, Canada.

A 39 year old G2P1 woman of Indian background presented at our Genetics Clinic for a positive Integrated Prenatal Screen (IPS). The risk was 1 in 70 for the baby to have Down Syndrome. Her family history was negative. The amniotic fluid chromosome result showed a female fetus with an extra small metacentric marker in 30% of cells examined. The marker appeared to be i(12p) by GTG Banding. However FISH analysis identified the marker to be an i(21q), resulting in mosaic tetrasomy 21. The karyotype was 47,XX,+i(21)(q10)/46,XX. Parental chromosomes were normal. Level II ultrasound and cardiac screen were normal. The patient terminated the pregnancy. While we are still awaiting the final autopsy report, the external examination showed dysmorphic features not particularly in keeping with a diagnosis of Down Syndrome. Mosaic i(21q) is rare. Early reports (1980s) were likely misdiagnosed cases of mosaic tetrasomy 12p (Pallister-Killian Syndrome). To our knowledge two prenatal cases have been reported previously and only one (Nagarsheth and Mootabar 1997) was confirmed with FISH. This pregnancy resulted in a live-born with features of Down Syndrome. Due to the rarity and the controversy with the few reported live-born cases, the clinical picture of mosaic tetrasomy 21 is not well defined. Our case is of interest because it was detected through a positive IPS screen for DS and to our knowledge it is only the second prenatal case confirmed by FISH. The autopsy findings will add to the clinical picture of mosaic tetrasomy 21. A review of the literature and the autopsy findings will be presented.

Dynamic differential expression of *Paraoxonase-1 (Pon1)* in murine liver. L. Parker-Katirae^{1,2}, K. Nakabayashi¹, S.W. Scherer^{1,2}. 1) Genetics and Genomic Biology, Hospital for Sick Children, Toronto, Ontario, Canada; 2) Department of Medical and Molecular Genetics, University of Toronto, Toronto, Canada.

Differential allelic expression of specific genes, characterized by a preference for one allele in a non-parent of origin manner has been observed in maize, mouse and humans. This phenomenon has also been implicated in disease pathogenesis. We describe the expression pattern of murine *Paraoxonase-1 (Pon1)*, a gene involved in lipid metabolism and implicated in the formation of atherosclerotic lesions. *Pon1* is member of a family of at least three genes that are found in a cluster on chromosome 5 and 7 in mouse and human, respectively. Previous studies have shown that *Pon2* and *Pon3* display preferential maternal expression in mouse. *Pon1* is adjacent to *Ppp1r9a*, a gene recently shown to be imprinted in both human and mouse. Using quantitative methods, we measured the expression of *Pon1* in liver at different stages of embryonic development using F1 hybrid crosses between C57BL/6J (B) and CAST/Ei (C), and C57BL/6J (B) and JF1/Ms (J). At the same time, SNaPshot analyses were performed to quantify the frequency of both parental alleles. At 12.5 days post coitum, a frequency of 61% and 89% of the C allele was observed in BxC and CxB crosses, respectively. The frequency of the J allele was measured at roughly 95% in both JxB and BxJ. However, at P0, the frequency of the C allele decreased to approximately 24% in BxC and CxB hybrids, whereas the frequency of the JF1/Ms was an average of 62% in JxB and BxJ livers. We concluded that *Pon1* shows a non parent-of-origin allelic preference in its expression, with variations throughout development. To our knowledge, this is the first case of a dynamic differential allelic expression pattern documented to date.

Congenital diaphragmatic hernia in WAGR syndrome: additional evidence that *WT1* plays an important role in diaphragm development. *D.A. Scott*^{1,2}, *M.L. Cooper*¹, *P. Stankiewicz*¹, *A. Patel*¹, *L. Potocki*^{1,2}, *S.W. Cheung*¹. 1) Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Texas Children's Hospital, Houston, TX.

WAGR (Wilms tumor, Aniridia, Genitourinary anomalies and mental Retardation) syndrome is a contiguous gene deletion syndrome involving *WT1*, *PAX6* and possibly other genes on chromosome 11p13. *WT1* is required for normal formation of the genitourinary system and the high incidence of Wilms tumor and genitourinary anomalies found in patients with WAGR are attributed to haploinsufficiency of this gene. It has been hypothesized that *WT1* also plays an important role in the development of the diaphragm. During mammalian embryonic development, *WT1* is expressed in the pleural and abdominal mesothelium that helps to form the diaphragm and mice that are homozygous for a deletion in the mouse homolog of *WT1* have diaphragmatic hernias. Case reports describing congenital diaphragmatic hernias in infants with Denys-Drash and Frasier syndromes, both of which are caused by mutations in *WT1*, provide additional support for this hypothesis. We report an infant with aniridia, bilateral cryptorchidism, vesicoureteral reflux, and a right-sided diaphragmatic hernia. G-banded chromosome analysis revealed a deletion of 11p13-p15.3. Breakpoint regions were refined by FISH and deletion of the WAGR critical region, including *WT1*, was confirmed. A complete description of this patient's cytogenetic abnormality will be provided. A review of the medical literature identified a second patient with a deletion of 11p13, a left-sided congenital diaphragmatic hernia, and anomalies that may be consistent with a diagnosis of WAGR including bilateral microphthalmia, a small penis, bilateral cryptorchidism, and a hypoplastic scrotum. These cases support the hypothesis that *WT1* plays an important role in diaphragm development and suggest that deletions of *WT1* may predispose individuals to develop congenital diaphragmatic hernia.

DNA damage detection by comet assay in personnel occupationally exposed to ionizing radiation. Evidences of adaptative response. A. Corona-Rivera¹, T.A. Garcia-Cobian¹, I. Ceja-Andrade¹, R. Troyo-San Roman¹, E. Castillo-Corona², L. Bobadilla-Morales¹. 1) Lab de Genetica Humana, Univ Guadalajara, CUCS, Guadalajara, Mexico; 2) Instituto Jaliscience de Cancerologia.

Ionizing radiation is a known genotoxic agent due to the induction of breakages in the DNA strand. The hospital personnel occupationally exposed to ionizing radiation (POE) do not have to exceed annual dose of 50 mSv. The detection of low dose biological effect of ionizing radiation in POE is based on the improvement of methods to detect genome integrity damage. Our goal was to evaluate comet assay as suitable biomonitoring tool in POE exposed to low doses of ionizing radiation. The study group was constituted by 7 POE from radiotherapy and nuclear medicine, and controls matched by age and sex. Comet assay was performed from blood samples in four conditions: uncultured cells unirradiated and irradiated to 1.5 Gy, and cultured cells (72 hs) unirradiated and irradiated to 1.5 Gy. 50 images from condition were captured, visual analysis by categories proposed by Avishai (2003) was used. In POE group annual registered dose was below to 10 mSv. Damage differences were not observed in unirradiated cell conditions in POE versus controls. With respect to irradiated cells, comparison POE versus control in 72 h cultures, were not observed differences. With respect to the evaluation of comet assay in uncultured conditions was observed: (1) in unirradiated blood samples the POE group showed a non significant increase of damage versus controls, (2) in irradiated blood samples differences were not observed between POE and controls, (3) in POE comparison basal versus irradiated, was observed damage increase only in category III comets, and (4) in control comparison basal versus irradiated, significant increase of damage was observed in all the measurements. These results suggest the existence of activation of damage repair mechanisms and adaptative response in POE. Comet assay is able to detect acute damage. Such method is suitable as biomonitor to the effect of low dose radiations in risk groups.

***FMR1* repeat length in premature ovarian failure and mothers of trisomy.** K. Bretherick¹, M. Fluker^{2,3}, M. Stephenson³, W. Robinson¹. 1) Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 2) Genesis Fertility Center, Vancouver, BC, Canada; 3) Department of Obstetrics and Gynaecology, BC Children's and Women's Hospital, Vancouver, BC, Canada.

Advanced maternal age is the only known risk factor for having a trisomic pregnancy. Reduced follicular pool size or physiologic changes in aging ovaries may be responsible for the increase in risk with age. Premature ovarian failure (POF) is the occurrence of menopause before the age of 40 and can be due to either ovarian follicular depletion or dysfunction. It is therefore possible that genetic factors that predispose to POF are also associated with an increased risk of having a trisomic conceptus. The most significant single gene association with POF is with *FMR1* premutation size alleles (60 repeats), which are found in up to 7% of women experiencing POF. We therefore examined *FMR1* repeat length by fluorescent PCR and chemiluminescent Southern Blot in 196 women who had experienced a trisomic pregnancy, 47 women with POF, and 107 control women. We found no premutation size alleles in our population of trisomy mothers, and the allele distribution of this group did not differ significantly from that of the control population. Our sample of 47 POF women had 2 premutation size alleles, a result consistent with that in the literature. Interestingly, there was an excess of alleles 33 repeats in our POF population as compared to controls (18.1% in POFs vs 7.5% in controls, $p=0.006$), a finding that has not been previously reported. This excess of alleles 33 repeats was not present in our sample of trisomy mothers. We assessed *FMR1* repeat length weighted by X chromosome inactivation ratio in our POF and control populations. While *FMR1* repeat size weighted by inactivation ratio was also associated with POF ($p=0.01$), it was not more significant than repeat size alone. In conclusion, our results suggest that the *FMR1* gene is not associated with an increased risk for trisomic pregnancy. However, the unexpected finding that *FMR1* alleles of 33 repeats may be associated with an increased risk of POF is of potential clinical importance.

Disruption of DMD and deletion of FACL4 causing developmental delay, hypotonia, and multiple congenital anomalies. *S. Bhat*¹, *S. Ladd*¹, *K. Schmidt*², *C.E. Schwartz*¹, *B.R. DuPont*¹, *R.E. Stevenson*¹, *A.K. Srivastava*¹. 1) J. C. Self Research Institute of Human Genetics, Greenwood Genetic Center, Greenwood, SC; 2) Department of Pediatrics, East Carolina University, Greenville, NC.

Defects in genes located on the X-chromosome are responsible for the causation of mental retardation (MR) in males. Several X-linked syndromic and nonsyndromic MR genes have been identified utilizing X chromosomal rearrangements associated with MR. We have studied a 3-year-old boy with limited speech, growth failure, hypotonia, microcephaly, multiple congenital anomalies, significant developmental delay and a karyotype reported to be 46, Y, inv(X)(p11.4q13). He also had ASD and VSD that were surgically repaired. The brain magnetic resonance imaging was normal. The molecular analysis of the inversion breakpoints allowed us to locate the Xp-breakpoint in the Duchenne Muscular Dystrophy (DMD) gene. Several known genes mapped within the second breakpoint region at the Xq13, but none was found to be inactivated. FISH analysis using a probe from the Xq13 region led us to suspect the presence of an additional chromosomal rearrangement, telomeric to the Xq13 inversion breakpoint. We identified a deletion at Xq22.3. Using FISH and STS analyses, we mapped the deleted region between markers DXS1413 (cen.) and DXS456 (tel.). RT-PCR analysis further confirmed the deletion of GUCY2F, NXT2, KCNE1L, and FACL4 genes. Deletion of these genes has previously been reported in a contiguous gene deletion syndrome, ATS-MR (OMIM #300194), and mutations of FACL4 have been identified in patients with nonsyndromic XLMR. These findings will assist in developing an adequate care strategy for the patient who is likely to develop classic DMD due to the loss of DMD expression, and MR due to deletion of the FACL4 gene. It remains to be seen if the absence of a gene from the deleted region may have also contributed to the development of the ASD and VSD observed in the patient.

Fragile X Syndrome: Screening strategies in Indian context. *S. Padma, K.R. Manjunatha, S.R. Girimaji*, G.K. Chetan, S. Roy, E. Venkataswamy, S. Balu.* Department of Human Genetics & Psychiatry*, National Institute of Mental Health & Neurosciences (NIMHANS), Bangalore-560029, India.

Fragile X syndrome represents the most common inherited cause of mental retardation, second only to Down's syndrome with a prevalence of approximately 1 in 4000 males and 1 in 8000 females. The key clinical features of the fragile X-syndrome in males are mental retardation, elongated triangular face, lop ears and macro-orchidism. The fragile X syndrome was the first disease shown to be associated with "dynamic mutations" caused by an amplification of an unstable CGG repeat sequence located at the 5' untranslated region of FMR1 gene. A fragile site at the distal long arm of the X chromosome (FraXq 27.3) is the hallmark cytogenetic feature of the syndrome. AIM: This study was aimed to facilitate both cytogenetic and molecular genetic screening of fragile X syndrome in Indian population for the analysis of the mutation. SUBJECTS AND METHODS: Fifty subjects with fragile X syndrome clinical features, were studied. Cytogenetic and molecular genetic analysis using lymphocyte culture, PCR and Southern technique, were done. The PCR method provided rapid and reliable results for the identification of fragile X negative and positive patients. The PCR-positive cases were confirmed by the CGG repeat expansion on Southern blot analysis with a positive cytogenetic result, their methylation status was also known which helps in finding out the mosaic individuals. CGG polymorphism in normal individual was studied using radioactive PCR and the most frequent repeats in Indian population was found to be 31 in the present analysis. CONCLUSIONS: The cytogenetic and molecular genetic techniques improves diagnostic accuracy and allows to verify the normal condition of FRAXA locus, identify carriers, detect complete mutations and also mosaics in fragile X syndrome for giving proper genetic counseling in fragile X families who are at high risk of having the affected gene. With fragile X syndrome being such a complex condition, national screening would need to be considered for families before a child is conceived so that an informed choice can be made. Email: padmaradha@yahoo.com.

Report of a 46,X,t(X;1)(q22.1;p34.1) female with short stature, dysmorphic features and multiple congenital anomalies. *R.K. Pettman¹, C. Lee³, J.P. Welch³, M. Ludman^{1,2}, S. Dyack^{1,2}.* 1) Medical Genetics, IWK Health Centre, Halifax, NS, Canada; 2) Department of Pediatrics, IWK Health Centre & Dalhousie University; 3) Department of Pathology & Laboratory Medicine, IWK Health Centre.

We present a 4-year-old female with short stature, dysmorphic features including downturned corners of her mouth and epicanthic folds, and multiple congenital anomalies including an ASD, left cystic kidney, umbilical hernia, prominent fingertip pads, bilateral colobomas and talipes. This constellation of features is not known to be those of an identifiable syndrome. Cytogenetic studies revealed a balanced de novo translocation 46,X,t(X;1)(q22.1;p34.1). X-inactivation studies were performed twice and revealed the derivative X chromosome containing XIST being inactivated in at least 10% of the cells, resulting in a partial functional disomy Xq and a possible partial monosomy 1p. X-inactivation is typically non-random in balanced X:autosome translocations, with inactivation of the intact X chromosome. Random X-inactivation with X:autosome translocations have been reported. Most had an X-chromosome breakpoint between Xq28->Xqter, while our probands breakpoint was more proximal at Xq22.1. Four reported cases with a breakpoint in proximal Xq had incomplete skewing of X-inactivation with the derivative X inactivated in only 2-5% of cells. This patient had a significantly greater inactivation ratio than these reported cases. The possible contributors to the development of this phenotype include disruption or deletion of a gene, or genes, at the translocation breakpoint(s), partial monosomy 1p and functional disomy Xq22.1->Xqter. Review of the literature made a disruption or deletion less likely. Some of our patient's features have each been reported in partial monosomy 1p, but they are not often found in the same individual. Males with duplications involving Xq display a consistent phenotype, which includes many of our patient's features. This suggests that partial monosomy 1p may play a role in the development of this phenotype; however, it is more likely that functional disomy Xq in a proportion of her cells is the major contributor to the development of this unusual phenotype.

A novel satellite DNA family generated by microdissection of Y-chromosome of Indian muntjac (*M. muntjac vaginalis*). Y.C. Li¹, Y.M. Cheng², L.J. Hsieh², S.J. Liao², S.Y. Chang², C.C. Lin². 1) Chung Shan Medical Univ, Taichung, Taiwan; 2) China Medical Univ Hosp, Taichung, Taiwan.

Cytogenetically, the Indian muntjac is the most fascinating mammalian species with only 6 chromosomes in female and 7 chromosomes in male. Extensive tandem fusions and few centric fusions occurred in the ancestor species with $2n=70$ resembling the *Mazama gouazoubira* and *Hydropotes inermis* which karyotype were thought to have resulting the present-day Indian muntjac karyotype. The different chromosome number between male and female of the species is due to the fact that the X-chromosome centric fusions with autosome 3 to forming the X+3 chromosome and therefore the female have two X+3 chromosomes. The male on the other hand has one X+3 chromosome, an unfused chromosome 3 and a Y-chromosome, thus having one more chromosome than the female. Satellite DNA appeared to play an important role in karyotypic evolution of the mammalian species by promoting chromosomal rearrangement. In this study, we isolated a DNA clone, designated MMV-0.4 using a chromosome microdissection technique from the Y-chromosome of Indian muntjac. By screening microclones constructed from DOP-PCR products initially generated from 15 whole Y chromosome, with cervid satellite I, II and IV, a microclones (IM-Y5-7) negative for all three satellite DNAs was obtained. The size of the clones is 426-bp and appeared to contain a ~400-bp monomer. Southern blot analysis showed tandem repetitive nature of the clone with polymeric ladder signal of ~400-bp. No similar sequence of the clones have been deposited in the GenBank data base suggesting that they could belong to a new cervid satellite DNA family with monomer size ~400-bp. The clone hybridized specifically to the heterochromatin portion of the Y chromosome and the pericentric region of the chromosome 3 of the male Indian muntjac as demonstrated by FISH. The solely present of MMV-0.4 in the Y-chromosome and chromosome 3 could provide a mechanism for the formation X+3 chromosome (by close association of the X-Y and chromosome 3) of the Indian muntjac. The study was supported by grants from the NHRI (NHRI-EX92-9207SI) and from the NSC (NSC92-2320-B-040-048), Taiwan.

Familial Subtelomere Rearrangement Determined via Multiplex Ligation-Dependent Probe Amplification (MLPA) and Fluorescent In-situ Hybridization (FISH). *H. Rishag, D. Cohn, K. Lee, S. Skinner, M.J. Friez, B.R. DuPont.* Greenwood Genetic Center, Greenwood, SC.

A 9 year-old white male was noted to have developmental delay with autistic features. He was the product of a twin pregnancy with premature labor and delivery at 34 weeks gestation. He failed to grow during the last 2 weeks of the pregnancy while his fraternal twin continued a normal growth pattern. In the newborn period he had feeding problems, reflux, dependent edema, and colic. Surgery was done for an atrial septal defect, bilateral inguinal hernias, and bilateral strabismus. By 2 years of age, he exhibited global developmental delays, withdrawn behavior, and limited dysarthric speech. Dysmorphic features included: plagiocephaly, low posterior hairline, hypoplastic midface, smooth philtrum, posteriorly rotated and low set ears, upslanting palpebral fissures, long eye lashes, telecanthus, thin lips, single transverse palmar crease on the right and bridged palmar crease on the left hand, stiff fingers and slight bilateral 5th finger clinodactyly. He was noted to have an extensive family history for mental retardation and birth defects. Furthermore, his mother has had four 1st trimester miscarriages. High resolution chromosome analysis revealed an apparently normal male karyotype. However, subtelomere analysis, done by multiplex ligation-dependent probe amplification (MLPA), revealed abnormal findings consistent with a duplication of the subtelomeric region of chromosome 4p. FISH showed the additional 4p material to be on the end of the p arm of chromosome 22, ish der(22)t(4;22)(p16.3;p13)(D4S3360 +). Parental FISH analysis revealed the mother to have a balanced form of the translocation. Further studies on additional family members will be discussed, as well as, the use of MLPA as a diagnostic tool for identifying subtelomere deletions and duplications in combination with FISH based-testing.

Transcription factor gene expression changes and interaction networks in regenerating inner ear hair cells. *S. Bashiardes, D. Hawkins, R. Veile, V. Bhonagiri, M. Warchol, M. Lovett.* Washington University, School of Medicine, St. Louis, MO.

Much of human hearing loss is caused by loss of auditory hair cell (HC) function. Mammals cannot regenerate these essential mechano-electrical transducers of sound. However, birds have retained the ability to regenerate HCs from surrounding supporting cells. We have conducted the first large scale study of gene expression on chicken HCs as they regenerate. Pure sensory epithelia (SE), consisting of HCs plus supporting cells, were damaged with either neomycin or laser treatment. As the HCs regenerated, the cultures were sampled at various timepoints. Changes in gene expression were compared to undamaged control cultures on a microarray that interrogates the vast majority of transcription factor (TF) genes. These experiments involved multiple biological samples and hundreds of microarray comparisons. Gene expression changes in SEs from the cochlea (auditory HCs) and the utricle (vestibular HCs) were measured, allowing us to identify changes that were specific to the treatments, to the SE type, or that were common to both. For example, 30 TF genes were reproducibly found to change during utricle HC regeneration. Among these were members of known pathways such as Jun/Fos and Hedgehog signaling, as well as members of unknown pathways such as Cebpg. We identified 77 genes that are specific to cochlea HC regeneration, including Foxh1, Znf174 and Hoxc10. Interestingly, only 5 TFs show similar changes in both SE types under all treatments and timecourses. These experiments were also conducted on neomycin damaged mouse SE to identify TF changes that occur in an SE that is incapable of regenerating. To test the relevance of specific TFs, we employed RNAi on regenerating SEs to knockdown specific TFs and measure the effect on regeneration, as well as on the expression of all other TFs. RNAi on over a dozen TFs indicates that 3-fold knockdowns can be routinely achieved, and that most of these do not appreciably affect regeneration. However, specific TF knockdowns do arrest regeneration. In these ways we have begun to identify the important TF pathways in avian HC regeneration.

Large scale gene expression profiles of early mouse inner ear development. *S. Sajan, M. Lovett.* Department of Genetics, Washington University, St Louis, MO.

Hearing loss affects ~30 million Americans, one-third of those being between the ages of 21 and 65. Environmental factors play a role, but up to half of all hearing loss is attributable to some form(s) of genetic abnormality. Sensorineural deafness, deafness due to damage to the sensory hair cells and/or the nerves of the inner ear, is the most common type of hearing loss. The inner ear contains the auditory (cochlea) and the vestibular organs which detect sound and movement, respectively. These structures contain many cell types including hair cells, the mechanosensory transducers of sound or motion. However, only a handful of genes are known to play a role during inner ear development, partly because these structures are small, complex and inaccessible. The majority of inner ear developmental genetics has yet to be explored. To expand our understanding of this important organ and to obtain a base line measurement of normal gene expression, we micro-dissected inner ears from mouse embryos at stages E9 to E15 in half-day intervals (covering the earliest discernible structures of the inner ear up until the development of true hair cells). Gene expression profiles were derived using mouse Affymetrix gene chips. Two litters per developmental stage were obtained, the structures were dissected and duplicate gene-chips were hybridized. Genes were selected that were reproducibly differentially expressed by at least 1.5-fold between any two stages, and self-organizing maps created to identify genes with similar expression patterns. Approximately 600 genes were differentially expressed across the time course, of which 10% had previously been shown to be expressed in the inner ear. Genes with high expression at early stages (E9-E9.5) included *Dachshund 2*, *Choroidermia*, *Kinesin family member 1B*, and *NeuroD6*. Among those that are highly expressed later at E13.5 and beyond are *GATA3*, *FGF-20*, *Aryl hydrocarbon receptor*, *Neuronatin*, and *Otogelin*. Many of these observations have been validated by quantitative-PCR and/or in-situ hybridizations. This study provides new candidate pathways for further investigation in inner ear development and the genetics of hearing loss.

Frizzled Wnt receptor gene expression strongly associated with initiation of hematopoietic stem cell neural-transdifferentiation in vitro. *H. Hao, P. Wooley.* Orthopedics Surgery, Wayne State University, School of Medicine Detroit, MI.

Hematopoietic stem cell (HSC), an alternative source to provide pluripotent precursors, have been recognized to give rise to osteocytes, chondrocytes, hepatocytes, endothelial, adipose, pancreatic islet cells as well as neural cells. Our previously reported results indicate neural cells can be generated from HSC. However, the major genes that initiate and govern final cellular phenotypes remain unknown. To gain molecular understanding of neurogenesis of HSC, gene expression profiles were analyzed using cDNA microarray representing 11000 mRNAs in non-induced HSC (CD34+) and neural-transdifferentiated cells (nestin+) from the same hematopoietic isolate. The significant alterations in Wnt/Frizzled (Fz) gene expression levels were confirmed by reverse transcription PCR. Genes that differently expressed in nestin+ and CD34+ cells were identified. Wnt/Fz (40) and adhesion molecule (56) genes were up-regulated respectively (2 to 4 times increased in CD34+ cells). In nestin+ cell, Fz gene transient alteration strongly related to the neural induction media exposure. The up-regulated Wnt/Fz signal transduction related genes such as beta-catenin and dishevelled were also detected. However, Fz gene expressions were decreased and diminished after differentiated neural cell maker genes were present. This data is first time to suggest that the Wnt signaling pathway is critical for stem cell transdifferentiation, which will require functional testing. Our studies, therefore, provide not only a molecular basis to understand biological processes of stem cell transdifferentiation but also a useful model to explore the mechanism of Fz-beta-catenin expression in neurogenesis from atypical tissue source.

Analysis of neural-specific conditional deletions to identify the role of the paired-like transcription factor PITX2 in basal ganglia disorders. *D.M. Martin*^{1,2}, *J.M. Skidmore*¹, *A.M. Sciafani*¹, *P.J. Gage*³. 1) Pediatrics; 2) Human Genetics; 3) Ophthalmology, University of Michigan, Ann Arbor, MI.

The *Pitx* genes *Pitx2* and *Pitx3* are paired-like homeobox transcription factors expressed in the mammalian subthalamic nucleus and substantia nigra, respectively. These brain regions are part of the basal ganglia brain circuitry that controls movements. In mice, *Pitx2* is required for normal differentiation of subthalamic nucleus neurons, whereas *Pitx3* regulates survival of mesencephalic dopaminergic neurons. In mice, complete loss of *Pitx3* is associated with a Parkinsonian phenotype, whereas *Pitx2* deficiency is embryonic lethal, precluding analysis of the *Pitx2*^{-/-} neurological phenotype at later embryonic timepoints. Here we report a conditional deletion of *Pitx2* in the mouse central nervous system using Nestin-Cre transgenic mice. The Nestin-Cre transgene is expressed in neural progenitors in the brain, in the pituitary, and in craniofacial regions coincident with *Pitx2* expression. *Pitx2*^{flox/-}; Nestin-Cre transgenic mice are viable to e18.5, but are reduced in size and do not survive beyond the immediate postnatal period. Mutant embryos have an elongated nose, displaced eyes, fused mouth, and kinked tail. *Pitx2* mRNA and protein in the subthalamic nucleus are absent in *Pitx2*^{-/-} embryos, and reduced in *Pitx2*^{flox/-}; Nestin-Cre transgenic embryos, suggesting partial Cre-mediated recombination of *Pitx2* in developing subthalamic nucleus neurons. Ongoing analysis of these and other neural-specific *Pitx2* deficient mice will help define molecular mechanisms of *Pitx2*-mediated neuronal differentiation in developing subthalamic nucleus neurons, and clarify the role of *Pitx2* in movement and movement disorders.

PITX2 and LMX1B interactions in the developing mammalian subthalamic nucleus. *J.M. Skidmore¹, D.M. Martin^{1,2}*. 1) Pediatrics, University of Michigan, Ann Arbor, MI; 2) Human Genetics, University of Michigan, Ann Arbor, MI.

Understanding the mechanisms of regional specialization in the developing neuroepithelium is a central issue in developmental neurobiology. In the embryonic mouse forebrain and hindbrain, transcription factors including *Dlx*, *Pax*, and *Engrailed* genes function to specify distinct neuronal identities with specific neurotransmitter and anatomical phenotypes. *Pitx2*, a paired-like homeodomain transcription factor, is expressed in discrete regions of the developing mammalian brain. *Pitx2* is likely to have a role in regional specialization of distinct neuronal populations, given the recently identified requirement for *Pitx2* for normal development of neurons in the murine subthalamic nucleus (STN), a key component of basal ganglia and a target for deep brain stimulation in Parkinson's disease. The mechanisms regulating *Pitx2*-mediated STN development are not known. Here we report an analysis of STN gene expression in midgestation wildtype and *Pitx2* mutant mouse embryos, aimed at identifying potential downstream targets of *Pitx2* in this brain region. We found that *Pitx2* co-localizes at the cellular level with *Lmx1b*, a LIM-homeodomain transcription factor that also specifies *Pitx3*-expressing mesencephalic dopaminergic neurons in the developing mouse substantia nigra. We detected loss of LMX1B protein in the developing STN of *Pitx2* mutant embryos, indicating a potential genetic interaction between these two transcription factors. LMX1B expression is preserved in other brain regions of PITX2 overlap in *Pitx2* mutant mouse embryos, suggesting regionally specific interactions of *Pitx2* and *Lmx1b* in the STN during brain development. Ongoing studies will help clarify the precise molecular genetic pathways by which *Pitx2* and *Lmx1b* confer STN neuronal differentiation and lineage specification.

Role of Notch signaling in early skeletal development. *F. Engin*¹, *G. Zhou*¹, *Y. Chen*¹, *T. Honjo*², *H. Zheng*³, *B. Lee*^{1,4}. 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston , TX; 2) Department of Medical Chemistry, Graduate School of Medicine, Kyoto University, Kyoto, Japan; 3) Huffington Center of Aging, Baylor College of Medicine, Houston , TX; 4) Howard Huges Medical Institute, Houston, TX.

Evolutionary conserved Notch genes regulate cell fate determination, differentiation, proliferation and apoptosis in multiple developmental pathways. Defects in Notch pathway have been implicated in a variety of human diseases such as T cell leukemias, a type of cerebral arteriopathy (CADASIL), and a common form of congenital heart disease (Tetralogy of Fallot). However, the role of Notch signaling in early skeletal development is still poorly understood. Mutations of the ligands of Notch signaling in humans cause skeletal patterning defects including Alagille syndrome with abnormal butterfly vertebrae, and spondylocostal dysostosis characterized by vertebral defects, rib abnormalities, short trunk and dwarfism. We performed in vitro and in vivo studies to elucidate the function of Notch signalling during chondrogenesis. In transient transfection studies, Notch1 intracellular domain (NICD) was able to activate a type II collagen (Col2a1) reporter construct by itself, as well as synergistically with SOX9. Presenilin 1 and 2 (PS1/PS2) liberate the NICD from the membrane. Interestingly, a chondrocyte specific double knock out of (PS1/PS2) showed that these mice appeared to be normal at birth, but then gradually exhibited shortened axial and appendicular skeletons, without obvious segmentation defects. Transgenic mice over-expressing Notch1 ICD in chondrocytes die shortly after birth due to severe chondrodysplasias reminiscent of the Sox5/6 double knock out. Histology analyses of these mice suggest that chondrogenesis was initiated but chondrocyte proliferation and maturation was severely blocked. To further understand the underlying molecular mechanism, we are currently performing analyses of chondrocyte specific markers on these transgenic mice. In conclusion, our data strongly suggest that Notch signaling has an important role during chondrogenesis perhaps by regulating the proliferation of this cell population.

***ITSN1* is essential for early brain development as shown in the *Itsn1* knockout mouse.** X-N. Chen, Y.W. Brown, J.R. Korenberg. Med Gen Inst, Cedars-Sinai Med Cntr, UCLA, Los Angeles.

ITSN1 maps to chromosome 21 and encodes a scaffolding protein regulating clathrin coated vesicles, some forms of receptor mediated endocytosis, mitogenic signaling and dendritic shape through its role in cdc42-mediated control of the actin cytoskeleton. We previously showed that the *Itsn1* deficient mouse exhibits dysgenesis of the cerebral cortex, absent midline brain structures and hippocampal malformation, which mirrors CNS defects in human deletion 21. We now show that these defects originate in early embryogenesis, are accompanied by prenatal mortality and implicate a dosage dependent role for *ITSN1* in CNS morphogenesis. We determined the viability and brain structures of *Itsn1* homozygous (-/-), heterozygous (+/-) and normal littermates (+/+) from E10.5 through P3. These included 91 embryos, 103 neonates, and 220 adult mice from 142 *Itsn1*^{+/+}, 209 *Itsn1*^{+/-}, and 63 *Itsn1*^{-/-}. We found the expected ratio of 1:2:1 from E10.5 to 15.5. Due to possibly decreased fitness in the *Itsn1*^{+/-}, the ratio of *Itsn1*^{-/-} to *Itsn1*^{+/+} was used as a more sensitive indicator of demise. This decreases to 0.6 at E16.5 (n=32), to 0.5 at P1 (n=38), to 0.3 at P3 (n=65) and to 0.31 in the adult (n=220). This suggests that *Itsn1* deletion causes embryonic lethality and increased neonatal and postnatal mortality. Using Hematoxylin & Eosin staining and immunohistochemistry with antibodies for Reelin and Dcx, we examined the development and numbers and distribution of Cajal-Retzius cells and radial organization of Dcx-positive elements of the preplate (PP), cortical plate (CP) of cortex. We observed abnormalities in the *Itsn1*^{-/-} vs the *+/+* from E13.5 including: 1) disoriented and malpositioned mitotic cells in all embryonic stages examined; 2) reduced number, abnormal distribution and morphology of Cajal-Retzius cells in the PP and CP; 3) increased Dcx-positive radial columns. Taken together, these results suggest that *Itsn1* deficiency adversely affects the migration and differentiation of Reelin- and Dcx-positive neurons and, combined with previous results (Pucharcos et al. 1999) that these cortical processes are mediated largely by the ubiquitously expressed short form of *ITSN1*.

Conservation of the visual system from flies to humans: *Drosophila* homologs of *Chx10*, a human microphthalmia gene essential for eye development. *T. Erclik*^{1,2}, *V. Hartenstein*³, *H. Lipshitz*^{1,2}, *R. McInnes*^{1,2}. 1) Developmental Biology, HSC, Toronto, ON; 2) Molecular and Medical Genetics, UofT, Toronto, ON; 3) MCD Biology, UCLA, Los Angeles, CA.

Chx10 encodes a homeodomain (HD) and CVC domain containing protein and is essential for mammalian eye development. In the mouse eye, *Chx10* is expressed in neuroretinal progenitor cells and bipolar interneurons. In mice, *Chx10* mutations lead to a hypocellular neuroretina, absent bipolar interneurons, microphthalmia and blindness; in humans, *Chx10* mutations cause an orthologous phenotype. We are using the powerful genetics of *Drosophila* to facilitate the dissection of the *Chx10* mammalian regulatory pathway. We have identified two *Drosophila* homologs of *Chx10*, *dChx1* and *dChx2*, which are >80% identical to *Chx10* in the HD and CVC domain. *dChx1* and *dChx2* map to the X chromosome where they are located 33 kb apart.

dChx1, but not *dChx2*, is expressed in the optic lobe of the fly embryo. In later stages of *Drosophila* development, *dChx1* and *dChx2* are co-expressed in the optic lobe. The fly optic lobe contains a class of interneurons that synapse with the retinal photoreceptor axons. Remarkably, *dChx* expression in these optic lobe interneurons suggests that fly visual system interneurons are orthologous to the *Chx10*-dependent bipolar interneurons in mammals. To determine whether the *dChx* genes are expressed in these fly optic lobe interneurons, we will use confocal microscopy to visualize the synaptic contacts of membrane labeled *dChx* neurons. We have identified the *dChx* optic lobe enhancer in a 9 kb fragment upstream of *dChx1* and are using this fragment to drive a membrane bound reporter.

To establish that the *dChx* genes are essential for visual system interneuron development in the fly, we have generated lethal loss-of-function mutants for *dChx1* and *dChx2* using transposon based deletions. Genetic screens will be performed to identify genes that enhance or suppress *dChx* mutant phenotypes. These genes and their mammalian homologs are likely to be members of a remarkably conserved genetic cascade regulating eye development from flies to vertebrates.

Molecular profiling of the transcriptomes of synthetic astrocytes differentiated from embryonic stem cells in vitro. A. Guha^{1,2}, D. Kamnasaran^{1,2}. 1) Surgery(Pediatric Neurosurgery), Hospital For Sick Children, Toronto, Ontario, Canada; 2) Surgery (Neurosurgery), Western Hospital, University of Toronto, Toronto, Ontario, Canada.

The pluripotency of Embryonic stem cells is determined by the ability to differentiate into derivatives of the three germ layers. We have determined an optimal protocol for the in vitro synthesis of astrocytes from murine embryonic stem cells harboring wildtype and p53 (+/-) genetic backgrounds. Immunocytochemistry with the best known marker for astrocytes, GFAP, showed expression in over 90% of cells. The majority of the astrocytes displayed morphologies similar to Type 1 astrocytes. cDNA microarray analyses on Affymetrix arrays were performed to provide detailed molecular profiling of the transcriptomes of these synthetic astrocytes. After normalizing and scaling of the data, the transcriptomes of synthetic astrocytes with wildtype or p53 (+/-) genetic backgrounds were found to be very similar ($r = 0.89$), with only 18 genes differentially expressed ($P < 0.001$). Immunocytochemistry showed these astrocytes were absent for the expression of OLIG2, NESTIN and OCT4, consistent with the microarray data. The transcriptomes of our synthetic astrocytes were compared to the published transcriptomes of in vivo astrocyte cultures established from various murine embryonic, post natal and adult brain sections (PNAS 101(22):8384-9). We calculated the extent of similarity (Pearsons correlation coefficient (r), $P < 0.001$) with possible astrocyte specific genes identified by having an expression pattern similar to GFAP, and the use a Recursive-Supervised Machine (R-SVM) class prediction analysis. Wildtype synthetic astrocytes were found most similar to astrocytes from the adult Corpus callosum ($r=0.281$), while synthetic p53 (+/-) astrocytes were most similar to astrocytes from the Cortex (P2) ($r=0.212$), by comparison with 325 astrocyte specific markers having expression similar to GFAP. By comparison with 156 astrocyte specific markers identified by R-SVM analysis, both wildtype and p53 (+/-) synthetic astrocytes were found to be most similar to astrocytes from the Cortex (P2) ($r=0.287$, $r=0.315$).

Program Nr: 1041 from the 2004 ASHG Annual Meeting

Characterization of *Prdm8*, a novel putative retinal transcription factor gene. C.C. Jung, I. Chau, R.R. McInnes.
Program in Developmental Biology, Hospital for Sick Children, Toronto, Ontario, Canada.

In mice and humans, disruptions to over 20 retinal transcription factors have been shown to result in developmental eye defects or retinal degenerations. We are interested in the molecular and genetic events underlying mammalian retinal development and maintenance, namely, the role of transcriptional regulators in guiding these processes. *PRDM8* (*PR-domain containing 8*) maps to 4q21 and is a putative transcription factor identified in a retinal cDNA screen for developmental regulatory molecules. *Prdm8* encodes a SET domain and several C2H2 zinc finger motifs. The SET domain is an evolutionarily conserved motif found among proteins that regulate chromatin structure. Disruption of SET domains often leads to impaired development or cancer. *Prdm8* homologues are found among all vertebrates genomes sequenced to date. In the adult mouse, full-length *Prdm8* is expressed abundantly in the retina and hippocampus, with smaller abundant mRNAs also in testis. *Prdm8* is broadly expressed in tissues that will contribute to the CNS and PNS. In the developing retina, antibody staining appears to be confined to post-mitotic cells. Preliminary results indicate that the Prdm8 protein is found in a perinuclear distribution in adult rod photoreceptors, whereas it is homogeneously distributed within nuclei of other retinal cell types. Based on expression data and homology to a family of developmentally important proteins, we predict that *Prdm8* may be required for neuronal development and that it may be influencing cell fate by regulating neuronal differentiation and/or maintenance. To identify the essential roles of *Prdm8* in the developing and mature brain, we have constructed loss-of-function *Prdm8* mouse mutants. To better understand the role of Prdm8 within the cell, a Yeast 2-hybrid screen is being performed to identify Prdm8 interacting proteins while a biochemical approach is being carried out to determine whether Prdm8 participates in histone modifications. These studies will define the role of *Prdm8* in the developing and mature retina, and address the significance of the heterogeneous nuclear distribution of Prdm8 among different classes of retinal neurons.

CNS-specific FGFR3 K644E transgenic mice: gene expression profile. *T. Lin*¹, *S.B. Sandusky*¹, *H. Xue*², *A.M. Heitman*¹, *M.S. Rao*², *C.A. Francomano*¹. 1) Laboratory of Genetics, NIA, NIH, Baltimore, MD; 2) Laboratory of Neurosciences, NIA, NIH, Baltimore, MD.

The K644E mutation in mouse FGFR3 results in constitutive activation of the receptor and is the cause of the neonatal lethal skeletal dysplasia, Thanatophoric dysplasia type II (TDII). Previously, we generated central nervous system (CNS)-specific TDII mice referred to as TDII-N. These mice demonstrated asymmetric changes in cortical thickness, dilated lateral ventricles, disorganized hippocampi, and cerebellar abnormalities, all of which correlate with brain abnormalities observed in human TDII patients. FGFR3 signaling operates along three pathways: PI-3 kinase, Ras-MAPK, and STAT signaling. To better understand gene regulation within and beyond these pathways, we examined brain RNA from both E12.5 and P0 TDII-N with cDNA microarray and RT-PCR assays. The expression pattern of several genes is altered in TDII mice when compared to WT littermates. Among these genes, an especially interesting one is Stat3, a key component in the STAT pathway that is involved in cell apoptosis regulation. Deletion of Stat3 is reported to increase cell proliferation in the myeloid lineage. Stat3 was found to be down-regulated in both embryo and P0 CNS. Lif interacts with Stat3 in primary neural progenitors and is also down-regulated in TDII E12.5 CNS. In the MAPK pathway, Mapk14 is up-regulated in E12.5 brain but down-regulated in P0 brain. Mapk14, also known as p38, is a member of the stress-activated protein kinase class of MAPKs, and plays a critical role in cell cycle control. We are currently quantifying additional genes that showed significant changes in microarray data. These include genes in FGFR3 and Stat1 signaling pathways as well as genes known to be important in cell cycle regulation and the developmental process of osteogenesis.

A mouse model for Amish lethal microcephaly. *M.J. Lindhurst¹, A. Chen¹, R.L. Nussbaum¹, D.M. Bodine², L.G. Biesecker¹.* 1) GDRB, NHGRI/NIH, Bethesda, MD; 2) GMBB, NHGRI/NIH, Bethesda, MD.

Amish lethal microcephaly (MCPHA) comprises severe microcephaly, a congenital malformation of the brain, increased urinary -ketoglutarate, and premature death. It is inherited in an autosomal recessive pattern. Previously, we reported a missense mutation in the *SLC25A19* gene encoding a mitochondrial membrane deoxynucleotide carrier, DNC, which segregates with the disease and alters a highly conserved amino acid. Functional analysis of the mutant protein using an *in vitro* transport assay revealed that nucleotide transport activity was lost. To understand how loss of function of this protein results in MCPHA, we have created a knockout mouse that has lost three exons of the *Dnc* gene. Homozygous mutant embryos fail to develop past 11.5 days p.c. Mutant embryos are small and most fail to complete neural tube closure in the mid and hindbrain portion of the embryo resulting in disorganization of developing brain structures. The neural folds have ruffled edges with convolutions extending into the neural tube of the trunk and tail. In addition, 10.5 day mutant embryos had very few erythrocytes in the circulation, indicating that yolk sac erythropoiesis requires DNC. Preliminary functional analyses of mitochondria from cultured cells isolated from 10.5 day embryos were unremarkable. Immunohistochemistry to both mitochondrial HSP70 and subunit I of the cytochrome C oxidase complex was normal in mutant cells. Quantitative PCR assays showed no change in mitochondrial DNA levels from whole embryos, yolk sacks or embryonic fibroblasts that were homozygous mutant. The presence of functional mitochondria in DNC deficient embryos indicates that DNC is not required to synthesize mitochondrial DNA or produce mitochondria. We hypothesize that MCPHA may be the result of the disruption of an undescribed non-mitochondrial function of DNC.

Imaging gene expression and anatomy in the developing human brain. *S. Lindsay¹, S. Sarma¹, M. Scott¹, J. Kerwin¹, J. Sharpe², T. Strachan¹, D. Davidson², L. Puellas³, S.C. Robson⁴, R.A. Baldock².* 1) Institute of Human Genetics, Univ Newcastle upon Tyne, Newcastle on Tyne, United Kingdom; 2) MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh, United Kingdom; 3) Dept. of Morphological Sciences, Faculty of Medicine, University of Murcia, Spain; 4) School of Surgical and Reproductive Sciences, University of Newcastle, Newcastle on Tyne.

Disorders involving abnormal brain development are common and can be severe. Identifying underlying causative genes and their expression patterns is important because it may reveal the tissue/cell type affected, genes which can potentially interact and possible targets for therapeutic interventions. Although the mouse is a powerful model system, there are differences between human and mouse transcriptomes [e.g. 1,2] and there is a clear need to study gene expression directly during human development. The MRC-Wellcome Human Developmental Biology Resource (HDBR) is a collection of intact, staged and karyotyped human fetuses, ranging from 4-12 weeks of development. The HDBR is held at the Institute of Human Genetics, Newcastle and the Institute of Child Health, London and developmental material is available to the international scientific community.

With material from the HDBR, we have used optical projection tomography [3], to generate 3D models of all stages of human development from Carnegie Stage 12 (CS12; 26 days of development) to CS23 (56 days of development). In addition to providing a means of analyzing and interpreting anatomical changes, our 3D models act as a framework onto which gene expression patterns are mapped. Visualizations of the 3D models and a prototype gene expression database are available on the web (<http://www.ncl.ac.uk/ihg/EADHB/>). The EADHB will be a valuable tool for neuroanatomical and gene expression studies of human development and is immediately useful as an educational resource for both clinicians and scientists. 1.Clement-Jones et al [2000] *Hum Mol Genet* 9: 695-7022. 2. Fougrousse et al [2000] *Hum. Mol. Genet.* 9: 165-1733. 3.Sharpe et al [2002] *Science* 296: 541-545.

Hippocampal hypocellularity during early development in the Ts65Dn mouse, an animal model of Down Syndrome. *H. Lorenzi, R.H. Reeves.* Dept Physiology, School of Medicine, Johns Hopkins University, Baltimore, MD.

Down syndrome (DS) is caused by trisomy of human chromosome 21 (HSA21). It is the most frequent genetic cause of mental retardation, affecting one in 800 live born human beings. Currently there are several animal models for DS. The Ts65Dn mouse is trisomic for the distal end of mouse chromosome 16 containing homologs of nearly half of the genes located on HSA21. Both DS patients and Ts65Dn mice have cognitive impairments involving hippocampal functions. In particular, Ts65Dn mice perform worse than euploid littermates in tasks that require spatial or working memory, such as the spatial version of the Morris Water Maze or the Radial Maze. The aim of this work is to study the hippocampus of Ts65Dn mice and their euploid littermates in early development and adulthood. We performed a quantitative study of the volume and number of neurons in the hippocampus of young (6 days old) and adult (3 months old) mice using unbiased stereology. Six-day old Ts65Dn mice presented a significant reduction in the number of granule cells (5.66×10^5 vs. 7.06×10^5 , $p=0.03$) and a non-significant 14% reduction in the number of pyramidal cells (9.34×10^5 vs. 10.8×10^5 ; $p=0.14$) compared to their euploid littermates. Accordingly, Ts65Dn mice showed a significant 19.4% decrease in the volume of the pyramidal cell layer (6.41×10^8 mm³ vs. 7.94×10^8 mm³, $p=0.04$) and a non-significant 14% reduction in the volume of the granule cell layer (1.68×10^8 mm³ vs. 1.95×10^8 mm³, $p=0.24$). We also identified an 18.2% reduction in the number of mitotic cells in the hilum, the region where granule cell precursors divide to generate the internal granule cell layer. Hypocellularity of the Ts65Dn hippocampus has origins early in development may contribute to the cognitive deficits in these mice.

Evidence that PDGF signaling is disrupted in human anomalies of the pulmonary veins. *S.B. Bleyl¹, Y. Saijoh¹, S.E. Klewer², J.C. Carey¹, G.C. Schoenwolf¹*. 1) University of Utah, Salt Lake City, UT; 2) University of Arizona, Tucson, AZ.

We mapped a gene for a congenital heart defect called total anomalous pulmonary venous return (TAPVR) to chromosome 4q12 in large Utah kindreds and identified two patients with missense mutations in the gene encoding the platelet derived growth factor receptor alpha (PDGFRA) and on a small paracentric inversion in the region. In TAPVR, the pulmonary vein fails to migrate to the left atrium during remodeling of the embryonic heart stalk. To examine the role PDGF signaling molecules play in TAPVR we examined their expression in the heart stalk of normal chick embryos using whole mount and section in situ hybridization. We found asymmetric expression of PDGFRA and its ligand PDGFA in the proliferating mesenchyme surrounding the developing pulmonary vein. Asymmetric proliferation of mesenchymal cells and differential left-right growth in the heart stalk are thought to be necessary for the displacement of the pulmonary vein to the left atrium. Based on our expression and genetic data, we hypothesize that right-sided expression and function of PDGFRA in the developing heart stalk is required for normal pulmonary vein development. In support of this hypothesis, we have observed TAPVR in chick embryos treated in ovo with specific PDGFRA function blocking agents. The effects of these agents on pulmonary vein development were assessed using vascular casting and 3D serial reconstructions. These studies will advance our understanding of the role of PDGF signaling during pulmonary vein and embryonic heart development and will provide insight about the role of PDGFRA in the pathogenesis of TAPVR.

Retinoid Antagonist in the Mouse: A New Experimental Model for Transposition of the Great Arteries. D.

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The morphogenetic mechanisms responsible for transposition of the great arteries (TGA) are still largely unknown, also because this malformation is very difficult to be experimentally reproduced. Retinoic acid (RA), the active metabolite of vitamin A, is an essential molecule for heart formation. The aim of the present study was to test the effect on murine heart morphogenesis of BMS189453 an antagonist of all RA receptors. It was administered at 5 mg/kg body weight (twice, at 10 h interval) to pregnant female mice at 6.5 (group 1), or 7.5 (group 2), or 8.5 (group 3) dpc. At birth the anatomical features were evaluated by stereomicroscopic examination. All mice of group 1 presented viscerio-atrial situs solitus with D-loop of the ventricles. The thymus was normal in 8 cases (80%) and ipoplastic in 2 cases (20%). Only 4 neonates (40%) presented TGA. 22 neonates (mothers treated at 7.5 dpc) presented viscerio-atrial situs solitus with D-loop of the ventricles. The thymus was abnormal in all but one mouse (absent in 5 cases); 6 mice (23%) had other types of extracardiac malformation, such as meroanencephalia. 15 mice (68%) presented various types of cardiac defects: 12 with TGA (54%) 2 with Truncus (9%) and 1 right aortic arch (5%). 20 neonates of group 3 presented normal cardiovascular anatomy, but altered thymus morphology. Resorbtion of the embryos due to the toxic action of the substance decreased significantly from 6.5 dpc to 8.5 dpc. All control mice presented normal extracardiac and cardiovascular anatomy. Conclusions: TGA can be consistently reproduced in the mouse by administration of a competitive antagonist of RA at 7.5 dpc that corresponds to 18 days of pregnancy in humans. This new experimental model for cardiac defects can be used for further dissection of the early molecular mechanisms of RA action, i.e. using microarray techniques and can be used in evaluating the function of genes related to RA.

Cells expressing a mutant of DNA polymerase are susceptible to chromosomal aberrations induced by an alkylating agent. *S. Mehra, S. Banerjee.* Department of Cancer Biology, Cleveland Clinic Foundation, Cleveland, OH.

Eukaryotic DNA polymerase (pol) is a DNA repair enzyme in base excision repair pathway (BER) and meiosis. Both wild-type (WT) and pol proteins are expressed in primary breast and colorectal tumors. A complex of truncated pol (pol) and X-ray cross complementing group 1, XRCC1 acts as a dominant-negative mutant. In this study, we investigated the potential role of pol in maintenance of chromosomal integrity. We used three cell lines derived from mouse embryonic fibroblasts of pol knockout and WT mice: (1) pol null cell line $-/-$, 19.4 (2) WT pol $+/+$, 16.3 and (3) pol $-/+$, 19.4P expressing pol (established in our laboratory). The cell lines were cultured and treated during the exponential growth with various doses (10nm to 100nm) of N-methyl-N-nitrosourea (MNU) for 1 hr and allowed to recover. Colcemid (50ng/ml final concentration) was added and after 3 hrs, cells were harvested, treated with hypotonic solution, fixed and stained by Giemsa. A minimum of 100 metaphases were examined for chromosomal aberrations such as radials, chromatid breaks and translocations. The average number of chromosome aberrations per cell was significantly enhanced in 19.4 cells treated with 25 nm MNU compared to the pol and WT cells. 19.4P cells were more resistant than 19.4 cells but still less resistant than 16.3 as the chromosome breaks could only be seen after treatment with 50nm and 100nm of MNU. 16.3 cells showed chromosomal aberrations when MNU concentration was increased to 100nm MNU. 19.4 cells were remarkably more sensitive to low doses of MNU than pol or WT. This may be due to an error-prone BER caused by pol deficiency leading to chromosomal aberrations.

Folic acid deficiency and/or methylenetetrahydrofolate reductase deficiency induce adverse reproductive outcome and congenital heart defects in mice. *D. Li¹, L. Pickell¹, Y. Liu¹, Q. Wu¹, J. Cohn², R. Rozen¹*. 1) medical genetics, Montreal Children's Hospital Research Institute, Montreal, QC, Canada; 2) Institut de Recherches Cliniques de Montreal, Montreal, QC, Canada.

By virtue of its importance for protein and nucleotide synthesis and methylation reactions, folate may crucially impact embryonic development. Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism. Clinical reports suggest that folate deficiency and mild MTHFR deficiency may be associated with pregnancy complications and birth defects. To further investigate this hypothesis, we fed *Mthfr* $+/+$ and $+/-$ female mice a control diet (CD) or folic acid deficient diet (FADD) post-weaning. Plasma homocysteine levels in CD-treated $+/-$ dams and FADD-treated dams ($+/+$ and $+/-$) were found to be at least 2-fold higher than those in CD-treated $+/+$ dams. On gestational day (GD) 14.5, a significant increase in the rate of embryonic resorption and delay, indicative of fetal loss and intrauterine growth retardation (IUGR), was observed in these hyperhomocysteinemic mice, compared to CD-treated $+/+$ dams. Of the viable embryos, no congenital heart defects (CHD) were found in embryos from CD-treated $+/+$ dams. In contrast, 6.5% from CD-treated $+/-$ dams, 23.1% from FADD-treated $+/+$ dams and 16.2% from FADD-treated $+/-$ dams showed CHD. These defects included ventricular septal defects, double outlet right ventricle and endocardial cushion defects. Most of the affected embryos also showed hypoplasia of the myocardium and a few embryos had heart failure. Examination of embryonic *Mthfr* genotype demonstrated no consistent correlation with IUGR or with heart defects. Our findings show that low maternal folate status and decreased maternal MTHFR increase the incidence of pregnancy complications and a range of CHD, supporting the benefit of folic acid supplementation.

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Expression of Irf6 and Tgfb3 mRNA in murine palate epithelium is not affected by dioxin. *B. Schutte, S. Goudy, M. Malik.* Dept Pediatrics, Univ Iowa, Iowa City, IA.

Isolated cleft lip and palate is a common disorder with a complex etiology. An ideal, genetically simple, model to study clefting is Van der Woude Syndrome (VWS). Interferon Regulatory Factor 6 (IRF6) is the transcription factor responsible for VWS and is expressed in the medial edge epithelium (MEE) on the leading edge of the palate just prior to palatal fusion. This pattern of expression is similar to Tgfb3, a growth factor that is necessary for palate development. In mice that lack Tgfb3, the palate fails to fuse, in part, because the MEE fails to dissolve. The failure of the MEE to dissolve is also seen in clefts induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin). Previous studies have shown that dioxin increases the level of TGFB3 protein in human palate cultures while the amount of mRNA decreases slightly. However, palate fusion in mice is 200 times more sensitive to dioxin, and the effect of dioxin on Tgfb3 and Irf6 in the MEE in the murine model is unknown. In this study we treated murine palates with dioxin in culture to study the effect on Tgf3 and Irf6 mRNA production in the MEE. As expected, palate cultures in dioxin-containing media did not fuse. Using Laser Capture Microdissection, we specifically removed the MEE from the palate shelves. Following dioxin treatment, we found no apparent difference in TgfB3 and Irf6 mRNA when compared to control palate cultures using RT-PCR. We conclude that dioxin-induced cleft palate is not caused by down regulation of transcription of TgfB3 or Irf6. Future studies will elucidate whether dioxin affects Irf6 function, possibly by inhibiting post-translational activation.

Control of enteric nervous system differentiation by endothelin 3 and SOX10. *N. Bondurand*^{1, 2}, *D. Natarajan*¹, *A. Barlow*¹, *N. Thapar*¹, *N. Lemort*², *V. Pachnis*¹. 1) Division of Molecular Neurobiology, National Institute of Medical Research, London; 2) Inserm U468, Hôpital Henri Mondor, Creteil, France.

Hirschsprungs disease (HSCR), or congenital aganglionosis, is a common disease of newborns affecting approximately 1 in 4500 live births. HSCR is characterised by failure of enteric ganglia to form, usually in the distal part of the colon; this results in lack of peristalsis and intestinal obstruction in the affected segment of the gut, which, if left untreated leads to death. Several genes encoding transcription factors and signalling molecules have been shown to play key roles in enteric nervous system (ENS) development. Among these are the G-protein coupled receptor-B, its ligand endothelin3 (ET3), and the transcription factor SOX10. To understand the mechanisms by which these factors control cell differentiation during enteric development, we previously developed a primary culture system which allows us to isolate, expand and follow the differentiation of multipotential progenitors of the murine ENS (i.e, EPCs) (Bondurand et al., *Development*, 2003, 130: 6387-6400). Here we report the use of this system to study SOX10 function in ENS neurogenesis: we demonstrate, using retrovirus infection, that constitutive SOX10 expression inhibits overt neuronal differentiation of these EPCs. On the basis of these results and previous observations showing that ET3 inhibits neuronal differentiation in mixed ENS cultures, we decided to test the possibility of an interaction between ET3 and SOX10. To this end, we performed a series of in vitro assays based on our culture system and in vivo experiments making use of spontaneous or induced mouse models of these genes (i.e, the lethal spotted, dominant megacolon and Sox10^{-/-} mice). Altogether, our results point out the existence of a genetic interaction between these two genes and indicate that ET3 is likely to operate upstream of SOX10 to contribute to the maintenance of multipotent progenitors and the regulation of ENS differentiation.

Comparison of Gene Expression Patterns Induced by Different C-terminal Isoforms of RET. *S.M. Myers, S.D. Andrew, D.S. Richardson, L.M. Mulligan.* Dept Pathology, Queen's Univ, Kingston, ON, Canada.

The proto-oncogene *RET* encodes a receptor tyrosine kinase that is involved in development of the kidney and neural crest lineages. Alternative splicing of the gene leads to three distinct C-terminal RET isoforms, RET9, RET43 and RET51. Functional differences between isoforms has been suggested by phenotypic differences in animal models expressing only single RET isoforms and by different isoform requirements for neuronal survival in culture. A difference in the onset of isoform transcripts during early human embryonic kidney development has been shown. These data suggest that each isoform may stimulate expression of a unique spectrum of target genes. In previous studies, we have used cDNA expression arrays to show that a broad range of genes may be modulated by both RET9 and RET51. However, we were not able to directly compare quantitative and qualitative differences in gene expression associated with each RET isoform. Here, we have used a doubly inducible RET isoform expression system in embryonic kidney 293 cells that allows us to regulate both RET expression and RET receptor dimerization and downstream signalling. We have used cDNA gene expression arrays to compare the nature and extent of relative gene expression induced by each of the RET isoforms. We have identified 67 genes with increased expression in the presence of one or all three RET isoforms. In preliminary analyses, we have confirmed 33 of these genes by qRT-PCR and, in some cases, by western blotting. Our data suggest that similar genes are modulated in response to each RET isoform and that few, if any, genes show a unique isoform specific induction pattern. It seems likely that RET isoform specific functional roles may be cell type specific or related to quantitative differences in target gene expression.

FOXL2 function in human and mouse development. *M. Uda*¹, *C. Ottolenghi*², *L. Crisponi*¹, *M. Deiana*¹, *M. Marongiu*¹, *A. Meloni*¹, *J.E. Garcia*², *A. Forabosco*³, *A. Cao*¹, *D. Schlessinger*², *G. Pilia*¹. 1) Istituto di Neurogenetica e Neurofarmacologia, Consiglio Nazionale delle Ricerche, c/o Ospedale Microcitemico, Via Jenner s/n, Cagliari, Italy 09121; 2) Laboratory of Genetics, National Institute on Aging, Baltimore, Maryland, USA 21224; 3) Medical Genetics, Department of Mother and Child, University of Modena and Reggio-Emilia, Policlinico, Via del Pozzo 71, Modena, Italy 41100.

FOXL2 mutations cause gonadal dysgenesis or premature ovarian failure (POF) in women, as well as eyelid/forehead dysmorphology in both sexes (the Blepharophimosis-Ptois-Epicanthus Inversus Syndrome, BPES). To understand better the role of the winged-helix forkhead transcription factor FOXL2 in development, we created homozygous mice lacking *Foxl2*. Males and females are smaller than wild type and show distinctive craniofacial morphology with upper eyelids absent, resembling features of human BPES. Furthermore, in mice as in humans, sterility is confined to females. Although heterozygous females were able to reproduce, preliminary results indicate subfertility, in at least one of three mice strains utilized. Phenotype analysis found that in addition to some skeletal dysmorphology, severe anomalies were confined to eyelids and ovary. Ovarian follicle development was abrogated at the primordial follicle stage. We are currently evaluating malformations further at different stages of mouse development, and have begun complementary biochemical approaches. To detect interacting proteins we carried out yeast two-hybrid screens with cDNA libraries from human adult ovary and 17.5 dpc mouse embryo, and have developed a FOXL2-specific antibody for immunoprecipitation studies in cells expressing FOXL2 from a transfected cDNA. Three putative interacting proteins are being characterized, providing tools to help identify target genes for FOXL2 in the critical follicle development that determines female reproductive lifespan.

Isolating New Genes In Adrenal Development. *S. Ching, E.R.B. McCabe, E. Vilain.* Dept Human Genetics, Univ California, Los Angeles, Los Angeles, CA.

In humans, abnormal development of the adrenal gland leads to severe adrenal insufficiency which is often fatal if untreated. Adrenal Hypoplasia Congenita (AHC) is an inherited disorder in which the development of the adrenal cortex is defective. It can be inherited as either an X-linked or autosomal recessive disorder, each characterized by a distinct histopathology. X-linked AHC is characterized by the cytomegalic persistence of fetal adrenal cortex that fails to differentiate into the adult zone. Affecting approximately 1 in 12,500 births, only about half of these cases can be explained by a mutation in the gene DAX-1, an unusual member of the orphan nuclear receptor superfamily, suggesting that there are additional genes involved in adrenal development. The purpose of these studies is to identify other genes involved in adrenal development, and therefore, candidates for cases of AHC not associated with DAX-1 mutations. During adrenal development in rodents, there is a period of dramatic reorganization of the adrenal cortex between 13.5 dpc and P0. We therefore isolated RNA from the adrenals of female mice at these timepoints to be used as probes for microarrayed genes and ESTs. Analysis of the microarray data produced a list of differentially expressed genes, from which several were chosen for further analysis. *Fzd2* and *SFRP2*, two genes involved in Wnt signaling, showed respective 4.0-fold and 13.9-fold increases between 13.5 dpc and P0. RT-PCR confirmed that there were highly significant differences in mRNA transcript levels between these two timepoints. *Fzd2* is a transmembrane receptor which binds to Wnt protein at the cell surface and transmits the signal intracellularly via cGMP and increasing levels of intracellular calcium. *SFRP2* modulates Wnt signaling by binding to extracellular Wnt proteins and preventing them from binding to Fzd receptors located at the cell surface. These results suggest that Wnt signaling may play a previously uncharacterized role in adrenal development.

A novel gene for truncus arteriosus type 1. *K. Heathcote¹, P. Syrris², C. Dalageorgou¹, M. Guy¹, M. Khetyar¹, L. Abushaban³, C. Braybrook⁴, P.J. Scambler⁴, M. Patton¹, A. Crosby¹, N.D. Carter¹.* 1) Clinical Developmental Science, St George's Hospital Medical School, London, United Kingdom; 2) Cobbold Laboratories, University College, London, United Kingdom; 3) Cardiology Department, Chest Hospital, Code 13110, Kuwait; 4) Molecular Medicine Unit, Institute of Child Health, London, United Kingdom.

We identified a large consanguineous family which contained six individuals affected with truncus arteriosus, a failure of the pulmonary artery and aorta to septate during cardiological development. Echocardiograms confirmed type 1 truncus arteriosus in the three living affected individuals and no cardiac abnormalities in their parents. A homozygosity mapping approach was employed to identify the locus involved. The locus mapped to a 6cM region of homozygosity on 8p21, with a maximum LOD score of 2.92 at D8S1820 (Mapmaker/Homoz).

Candidate genes in this region were sequenced, including all cardiac expressed genes and all novel genes. A novel gene was identified on the Ensembl database which is the human homolog of mouse *Nkx2.6*, a gene encoding a homeobox transcription factor expressed in the sinus venosa and the myocardium of the outflow tract in the developing mouse heart. A Phe151Leu mutation was identified in the homeodomain of this gene. All three affected individuals were homozygous for this mutation and all parents who had had an affected child were carriers of the mutation. In the mouse the *Nkx2.6* gene is expressed during embryonic development and not in the adult. We have not detected expression of the gene in human heart samples from adults, or in second trimester heart samples. Expression analysis is currently underway using a human embryonic heart northern blot.

The closely related transcription factor *Nkx2.5* is involved in septation of the heart chambers and is expressed at a similar time in mouse embryonic development. Mutations in human *Nkx2.5* have been associated with septal defects. Therefore it appears that the human homolog of mouse *Nkx2.6* is a good functional candidate gene that may play a role in septation of the cardiac outflow tract.

Expression of the IL-1 family gene in mouse preimplantation embryos and precursors of embryonic stem cells.
R.L.C. Tavares^{1,3}, S.H. Kim¹, Z.S. Sun², V. Freitas³, Z. Rosenwaks¹, K. Xu¹. 1) The Center for Reproductive Medicine and Infertility, Cornell University, New York, NY, USA; 2) Department of Pediatrics, Cornell University, New York, NY, USA; 3) Escola Paulista de Medicina, UNIFESP, Sao Paulo, SP, Brazil.

Genes of the IL-1 (Interleukin-1) family have been described as participating in cross talk between preimplantation embryos and endometrium during implantation, but their role in embryonic stem cell culture is still not clear. Our purpose was to study IL-1 family gene expression during mouse embryo development and throughout the conversion of the inner cell mass (ICM) to embryonic stem cells. We used the OCT-4 as a marker of the totipotential state of the ICM. Eighteen zygotes retrieved from 6 to 7-week-old mice were cultured in M16 medium under mineral oil, with 15 (83%) reaching the blastocyst stage. Two zona-free blastocysts were used as a positive control for Oct-4 expression. The remaining 13 blastocysts were cultured in 4 well dishes, previously prepared with 0.1% gelatin and 0.6 ml of conventional embryonic stem cell feeder medium. The medium was changed daily, and after 4 days, six inner cell mass outgrowths (ICM-O) were removed. Each was loaded individually into a 0.5 ml PCR tube for the RT-PCR. All RT-PCR and PCR reactions were run with negative controls. PCR products were analyzed by electrophoresis. Gene expression of OCT-4, b-actin and Interleukin-1 receptor antagonist (IL-1ra) was successfully detected using SuperScript First-Strand Synthesis System for RT-PCR. Our results showed that all 6 ICM-O samples were positive for OCT-4 and b actin, confirming that the cells analyzed were totipotential. One of the six ICM-Os was also positive for IL-1ra. The results suggest that the IL-1 family gene is expressed in totipotential mouse embryonic cells and may be involved in the process of conversion toward their becoming stem cells. Further experiments including other genes of the IL-1 family and more passages of ICM-O during the establishment of embryonic stem cell culture may provide more information for efforts to elucidate the mechanisms of cellular differentiation and implantation process in mouse.

Genomic, cDNA, and Embryonic Expression Analysis of the Zebrafish Ortholog of *IRF6*, the Causative Gene in Van der Woude and Popliteal Pterygium Syndromes. *J. Ben*¹, *E.W. Jabs*^{2,3}, *S.S. Chong*^{1,2}. 1) Pediatrics, Ob/Gyn, and Lab Medicine, National Univ Singapore & Hospital, SINGAPORE; 2) Pediatrics, McKusick-Nathans Institute of Genetic Medicine & Center for Craniofacial Development and Disorders, Johns Hopkins Medicine, Baltimore, USA; 3) Medicine, Plastic Surgery, Johns Hopkins Medicine, Baltimore, USA.

Mutations in the *Interferon Regulatory Factor 6 (IRF6)* gene have been identified in the autosomal dominant clefting disorders Van der Woude and popliteal pterygium syndromes. The *IRF* gene family consists of nine members encoding transcription factors that share a highly conserved helix-turn-helix DNA-binding domain and a less conserved protein-binding domain. Most IRFs regulate the expression of interferon- and - after viral infection, but the function of *IRF6* remains unknown. Using initial sequence information from a 5 prime EST, coupled with 5 prime and 3 prime RACE experiments, we have isolated a full length zebrafish *irf6* cDNA. It contains an open reading frame encoding a 492 amino acid protein, and a GT-rich 3 prime untranslated region. The encoded protein has a Smad-IRF interaction motif and a DNA-binding domain. Genomic analysis indicates that the zebrafish *irf6* gene consists of nine exons, of which four coding exon splice junctions are highly conserved with other vertebrate species. Using probes from the 5 prime untranslated region and upstream promoter of *irf6*, the gene was mapped to linkage group 22 using a radiation hybrid mapping panel. By *in situ* hybridization analysis of whole mounts and cryosections, we demonstrate that *irf6* is present as a maternal transcript, suggesting an important if not essential role in the early embryo. During gastrulation, *irf6* was expressed at forerunner cells. No expression was observed at the early somite stages. At the 17-somite stage, distinct expression was observed in the otic placode and Kupffers vesicle. During the pharyngula and hatch periods, *irf6* was expressed at the olfactory and otic placodes, and epithelial tissues of endodermal origin such as the mouth, pharynx and intestine. In 5-day old larvae, *irf6* was also expressed in the pharyngeal arch.

Human Developmental Disorders: Categories of Genes. *G.A. Bruns^{1,2}, R.E. Eisenman¹*. 1) Genetics Div, Children's Hosp, Boston, MA; 2) Dept of Pediatrics, Harvard Medical School, Boston, MA.

The OMIM Morbid Map was sequentially searched for inherited disorders with known gene mutations not associated with pathway enzymopathy to assess the different types of genes underlying developmental disorders. From more than 300 disorders meeting criteria and evaluated to date, the genes involved in 100 disorders with developmental phenotype were categorized. When mutation in a gene is associated with more than one syndrome, that gene was tabulated only once. Among the disorders evaluated, transcription factor mutations are the most common at 19 percent. These include: 5 HOX; 2 Forkhead family; 2 SOX family; 2 GATA binding protein genes; and loci for 4 tissue specific transcription factors. Sixteen mutations involve structural proteins, including members of the troponin-tropomyosin complex and IF filaments. There are 6 mutations of cell adhesion or gap junction proteins; 6 of fibroblast growth factors and FGF receptors; 4 of signal transduction molecules; 3 of receptor or protein tyrosine kinases/phosphatases; and 2 of Notch related cell fate decision proteins. Four disorders are due to alterations in solute carrier (SLC) loci and 3 to mutation in ATP binding cassette (ABC) proteins. Organelle trafficking protein and nuclear envelope protein alterations accounted for 4 and 2 disorders, respectively. Two mutations involve genes for members of the helicase superfamily; 3 loci encode neural cell neurotrophic or neural cell restricted proteins; 2 involve a GDP/GTP exchanger or RAS family GTPase activating protein; and 1 specifies a histidine triad family member. In 8 disorders, the mutation involves a novel protein, most with significant homology in lower organisms. The remainder, currently, are single category entities. The distribution of these mutations reflects the disorders evaluated to date, with additional analysis in progress. However, alterations in loci specifying transcription factors, signaling molecules and structural proteins are prominent. Although a significant fraction of loci in mammals are limited to higher Eukaryotes, the vast majority of those underlying the developmental disorders evaluated here encode sequences of ancient origin.

Changes in gene expression associated with NSDHL loss of function in mouse embryonic fibroblasts. D.

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Seven human disorders of post-squalene cholesterol biosynthesis have been described. Common features include growth and mental retardation, major malformations, skeletal defects, and increasing severity with prenatal lethality for disorders involving earlier steps in the pathway. We are using microarray analysis in a mutant mouse model to unravel the pathogenesis of these disorders. Initial studies have focused on *Bpa*^{1H}, a null allele of the X-linked *Nsdhl* gene encoding a sterol dehydrogenase involved in cholesterol biosynthesis. Heterozygous *Bpa*^{1H} females have skin and skeletal abnormalities with a distribution reflecting random X-inactivation, while hemizygous male embryos die before E9.5. To obtain a large, homogeneous population of *Bpa*^{1H} cells, we generated doubly heterozygous females carrying a *Bpa*^{1H} X chromosome and an X expressing a GFP transgene that undergoes random X-inactivation. Embryonic fibroblasts were cultured from *Bpa*^{1H} and wild type (wt) female embryos, and >99% pure populations of GFP-negative cells were isolated by FACS. Labeled cDNAs from parallel cultures of sorted *Bpa*^{1H} or wt cells grown in normal or lipid-depleted sera (LDS) were hybridized to microarrays containing 22,000 mouse genes. Among 44 genes that showed higher expression (p-value < 0.001) in the *Bpa*^{1H} vs wild type cells when grown in LDS, 12 function in cholesterol biosynthesis, 7 are involved in fatty acid synthesis, 2 (*Srebp2*, *Insig1*) encode sterol regulatory proteins and 2 (*Ldlr*, *StarD4*) are lipid transporters. Of the 21 remaining genes, 16 are known genes, some of which have been previously implicated in cholesterol homeostasis or lipid-mediated signaling (*Map17*, *Ctsd*, *Epb7.2*, *Mdk*), and 5 are anonymous cDNA clones. Further characterization of the expression patterns and functions of these novel genes may reveal additional roles of cholesterol in development.

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X-linked genes differentially regulated during testis and ovary maturation. *J.E. Garcia, D. Schlessinger, C. Ottolenghi.* Laboratory Genetics, NIA/NIH, Baltimore, MD.

Sex chromosomes carry some genes that are sex-determining and can be modified in expression by gene dosage mechanisms. Several groups, starting with Rice (1984), have hypothesized that X-chromosome-enriched genes should be either male- or female-specific, but not both. In invertebrates, the X-chromosome was shown to be enriched in female-specific genes, but reports for mammalian systems have been conflicting concerning the degree to which sex-specific genes are on the X, and if so, which sex is selectively represented. To clarify the situation, we used *in silico* and experimental analyses (RT-PCR, Real time-PCR, and *in situ* hybridization). Genome-wide EST mapping was consistent with an enrichment of either male- or gonad-specific genes on the X chromosome. Arguing in favor of gonad rather than male specificity, genes reported to be selectively expressed in adult testis are consistently found to be expressed in the newborn ovary as well. Our results modify previous suggestions in two categorical ways: 1) Rices hypothesis that is, that the X chromosome is enriched in genes with sexually dimorphic expression is not supported; and 2) some enrichment does occur for a group of genes that are expressed early in development in both male and female gonads, and are then specifically turned off at later stages of ovarian differentiation. This suggests a previously undetected mechanism, possibly related to progression through meiosis, that differentially regulates a cohort of sex-related genes in maturing mouse ovary and testis.

SUMO modification of the Chx10 microphthalmia transcription factor is dependent on Pias1. *A. Huang¹, R.L. Chow^{1,2}, B. Muskat¹, R.R. McInnes¹.* 1) developmental biology, hospital for sick children, toronto, Ontario, Canada; 2) Department of Biology, University of Victoria, PO Box 3020, Station CSC, Victoria, BC V8W 3N5, Canada.

The homeodomain transcription factor Chx10 is essential for the normal development of the human eye. Loss of Chx10 function in humans and mice leads to microphthalmia and blindness. We hypothesized that proteins that associate with Chx10 are also likely to be required for eye formation, and that identification of these Chx10 partners will provide insight into other hereditary ocular defects. Using the yeast two-hybrid screen, we identified the protein inhibitor of activated STAT1 (Pias1) as a Chx10-interacting protein. Pias1 is a nuclear receptor coregulator and is also known to function in protein SUMO modification as a small ubiquitin-like modifier E3 ligase. Direct protein-protein interactions between Chx10 and Pias1 were confirmed by glutathione s-transferase (GST) pull-down and co-immunoprecipitation. Immunohistochemical analysis of mouse retinas revealed that Chx10 and Pias1 colocalize in cells of the inner nuclear layer. Furthermore we found that SUMO modification of Chx10 in 293 cells is dependent on Pias1. Although Pias1 can physically interact with two naturally occurring Chx10 splice variants (19 and -19), Chx10 sumoylation was observed only in the Chx10(-19) variant. The Chx10 splicing event results in the presence or absence of a 19 amino acid stretch within the CVC domain, a highly conserved region adjacent to the homeodomain that contains the sumoylation consensus sequence, MKTE. Mutation analyses established that the MKTE sequence is essential for SUMO conjugation to Chx10(-19). In summary, we have identified Pias1 as a Chx10-interacting protein required for the splice variant-specific SUMO modification of Chx10. Current studies will determine how Pias1 interaction and SUMO modification affect both the transcriptional activity of Chx10 and its biological function in retinal development. Post-translational SUMO modification of Chx10 is likely to play an important role in normal eye development and may represent an important regulatory mechanism for other hereditary ocular diseases.

Investigating the Effects of NSDHL Deficiency on Placental Development. *F. Jiang¹, D. Cunningham¹, L. Humphries¹, G. Herman^{1, 2}.* 1) Center for Molecular and Human Genetics, Columbus Childrens Research Institute, Division of Human Cancer Genetics, Comprehensive Cancer Center, Columbus, OH; 2) Department of Pediatrics, The Ohio State University, Columbus, Ohio.

The *Nsdhl* gene, located on X-chromosome, encodes a sterol dehydrogenase involved in cholesterol biosynthesis. Mutations in this gene cause the male lethal phenotype in bare patches (Bpa) mice and human CHILD syndrome. Affected male mouse embryos die in mid-gestation and demonstrate a thinner, more compact and less vascularized labyrinth layer of the fetal placenta. To further characterize the placental defects, in situ hybridization was performed to examine the expression of placental markers, including giant cell markers pII and pIII, trophoblast marker 4311 and labyrinth markers *dlx3*, *mash2*, and *gcm1*. The results suggest that the mutant placentas are generally delayed and do not maintain *mash2* expression in the labyrinth layer as early as 9.5 dpc. More striking is the dramatic reduction in the number of fetal blood vessels in affected male placentas. In situ hybridization of allantois and endothelium markers indicates that there is a failure of invasion of extraembryonic mesoderm which normally forms the endothelial lining of blood vessels in the fetal labyrinth. Our previous data have shown that there is no significant difference in cholesterol or total sterol levels between normal and affected embryos, suggesting that the defects observed are not caused by a lack of sterols. We hypothesize that NSDHL deficiency is disturbing one or more signaling pathways in the developing placenta, resulting in the defects observed. Sonic hedgehog (Shh) is processed normally in mutant immortalized fibroblasts grown in normal or delipidated medium. *Ptch-lacZ* mice have been crossed with Bpa mice to examine in vivo hedgehog signaling via its receptor Patched. The expression pattern suggests an overall delay in development in embryos carrying the mutant *Nsdhl* allele. We are also performing Shh and Wnt signaling assays on normal and mutant cultured fibroblasts to see if NSDHL deficiency affects the cellular response to Shh and Wnt signaling.

Brachydactyly caused by loss of function of *sFrp2* signaling. *R. Morello, T. Bertin, K. Preuss, P. Hermanns, Y. Chen, B. Lee.* Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

We have examined potential components of the Wnt signaling pathway during joint and limb development. *sFrp2* belongs to the secreted frizzled related family of proteins that share a cysteine-rich domain (CRD) with the Wnt family of receptors, called frizzled. Since Wnt molecules bind the CRD domain of the frizzled receptors, the sFrps proteins can function as soluble receptors for Wnt thereby modulating its downstream signaling. *sFrp2* is highly expressed during mouse embryonic development of the eye, brain, neural tube, craniofacial mesenchyme, kidney, joints, testis, pancreas and sub-epithelial structures. We found that during mouse limb bud development, *sFrp2* is expressed in perichondrium of the digits at E13.5, in all limb joints and mesenchymal tissue surrounding them at E15.5 and then in mesenchyme lining ossified diaphyses of autopod elements. We generated *sFrp2* knock-out mice via homologous recombination. From F1 crosses we obtained *sFrp2* null mice at the expected mendelian ratios at weaning. *sFrp2*^{-/-} pups are grossly indistinguishable from their WT counterparts and thrive into adulthood. Both histological and functional analyses at 10 weeks of age have not showed any morphological defects or alteration of the organs analyzed. However, skeletal preparations of newborn mutant pups have revealed brachydactyly of the distal skeletal elements of the limb (including a delayed ossification in the second phalanx of the digits). Moreover, a selective hind-limb syndactyly, involving usually the 3rd and 4th digit, was also observed. The pattern of brachydactyly is reminiscent of brachydactyly type E in humans. These data point to the importance of Wnt signaling in the regulation of chondrocyte proliferation, perhaps similar to Tgfb signaling via GDF5 in humans and mice. Current studies are aimed at elucidating the mechanisms underlying the brachydactyly defect and the first demonstration of Wnt signaling-mediated regulation of interdigital mesenchymal apoptosis.

The role of LGL1 in alveolar development and recovery from arrested alveolar growth. *K. Nadeau*^{1,3}, *R. Jankov*^{4,5,6,7}, *K.A. Tanswell*^{4,5,6,7}, *N.B. Swezey*^{5,6,7}, *F. Kaplan*^{1,2,3}. 1) Dept Human Genetics, McGill U, QC, Can; 2) Dept. of Pediat, McGill U, QC, Can; 3) Mtl Child's Res Inst, QC, Can; 4) Lung Biol and Can Insts for Health Res Group in Lung Dev; 5) Hosp for Sick Child Res Inst, TO, ON, Can; 6) Dept. of Physiol, U of Toronto, ON, Can; 7) Dept. of Pediat, U of Toronto, ON, Can.

Bronchopulmonary dysplasia (BPD), a leading cause of morbidity in preterm infants, is characterized by inhibited lung growth and alveolarization. We cloned LGL1, a glucocorticoid-induced, developmentally regulated gene in lung mesenchyme, and showed that reduction of Igl1 inhibits lung branching. Maximal fetal expression of LGL1 is concordant with the onset of alveolar septation and augmented surfactant production. Beginning in late gestation and continuing after birth, secreted Igl1 is imported by epithelial cells. We therefore hypothesized that Igl1 would have an additional role in alveolarization. We report increasing expression of LGL1 mRNA and Igl1 protein from postnatal day (PN) 7 to PN 14. We show that at PN7, Igl1 concentrates at the tips of budding 2nd alveolar septa, suggesting that Igl1 may be required for postnatal alveolarization. Interestingly, at PN14 concentration of Igl1 at septal tips was restricted to the lung periphery, where post-septation alveolarization is believed to occur. We showed that LGL1 expression is severely reduced in two rat models of impaired alveolarization (BPD) generated by exposure of newborn rats to 60% O₂ for 2 wks or 95% O₂ for 1 wk. Recovery of animals exposed to 95% O₂ was associated with normalization of Igl1 levels. Surprisingly, when accelerated lung growth in recovering animals is prevented following injection of a soluble FGF receptor-1 (sequesters FGF-2), Igl1 levels rapidly increase. This suggests a potential regulatory feedback link between Igl1 and FGF-2, a key growth factor of lung development. We speculate that Igl1 is important for normal alveolarization, that deficiency of Igl1 contributes to the arrested alveolar partitioning observed in BPD, and that recovery is associated with normalization of Igl1 levels.

Nr0b1 is expressed and functional in the early embryo, but not in the extra-embryonic visceral endoderm. K.

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The cytomegalic form of adrenal hypoplasia congenita is an X-linked disease caused by mutations in NR0B1, which codes for DAX1. Failure to create a conventional knock-out mouse model led us to hypothesize that Nr0b1 is expressed earlier than predicted and with a novel function. Previously we reported Nr0b1 expression in ES cells and preimplantation embryos. Our purpose was to identify Nr0b1 expression and map its patterns throughout murine embryonic development and to investigate a functional role for Nr0b1 in early embryos. Immunohistochemistry, in situ hybridization and western blots were used to determine Nr0b1 expression in mouse embryos from E3 through E12. Expression of the following steroidogenic proteins was investigated: progesterone, estrogen (and) and androgen receptors; Wilms tumor 1; steroidogenic factor 1; and cytochrome P450_{sc}. Nr0b1 expression was confirmed by quantitative RT-PCR. ES cells were treated with Nr0b1-targeted siRNAs; the phenotype observed was confirmed using a known inhibitor of Nr0b1 expression and the cells stained with Nr0b1 antibody. Nr0b1 expression was observed at a critical boundary throughout embryonic development; specifically, it distinguishes between the embryo and its extra-embryonic visceral endoderm. Expression was also found in polar bodies, hypothesized to determine polarity in embryos. Nr0b1 did not appear to function in the steroidogenic pathway at this early developmental stage, as determined by absence of steroidogenic proteins. By utilizing siRNA knock-down in ES cells and observing differentiation of treated cells, we demonstrated functional relevance of Nr0b1 in early development. Based on the expression observed in embryonic development and the differentiation induced with loss of Nr0b1 expression, we hypothesize that Nr0b1 is critical in early embryonic development and plays a role different from that in steroidogenesis.

LGL1 transgenic mice have accelerated newborn alveolarization. *L. Oyewumi*^{1,2}, *F. Kaplan*^{3,4}, *N.B. Swezey*^{1,2,5}. 1) Lung Biol Research, Hosp Sick Children, Toronto, ON, Canada; 2) Dept Physiology, Univ of Toronto, Toronto, ON, Canada; 3) Dept Human Genetics, McGill Univ, Montreal, QC, Canada; 4) Dept Pediatrics, McGill Univ, Montreal, QC, Canada; 5) Dept Paediatrics, Univ of Toronto, Toronto, ON, Canada.

We previously cloned LGL1, a glucocorticoid- induced, developmentally regulated gene in rat lung mesenchyme. We have provided evidence that LGL1 plays an important role in lung branching morphogenesis during early gestation, based on the effects of oligodeoxynucleotide-induced reduction of LGL1 expression and of exogenously supplied recombinant lgl1 protein. Moreover, we found that lgl1 may be important for normal alveolarization, that deficiency of lgl1 is associated with the arrested alveolar partitioning observed in a hyperoxic rat model with some features of bronchopulmonary dysplasia, and that recovery is associated with normalization of lgl1 levels. We report here studies of a mouse expressing transgenic LGL1 driven by the (mesenchymal) h-versican promoter. Increased copies of the LGL1 transgene cause developmental changes in alveolarization of the postnatal mouse lung. At post-natal day (PND) 1 the structural maturation of the transgenic lung is accelerated, characterized by a marked increase in mesenchymal and secondary septal thinning and an increased number of sprouting secondary septa. By PND 4, sprouting secondary septa are numerous and the airspaces are smaller than non-transgenic littermates. Immunohistochemistry for elastin (a marker of secondary septal formation) was used to identify alveolar myofibroblasts within alveolar walls and septa. At this time, transgenic mice have more elastin expression at the tips of airspace septa. By PND 14, the two groups are indistinguishable in all these respects. We propose that LGL1 regulates the development of alveolar structures during postnatal lung development.

Human pre-implantation development characterized by unique transcript modulation. *R. Raja*¹, *A. Dobson*², *M. Abeyta*², *R. Reijo Pera*². 1) Arcturus Bioscience, 400 Logue Avenue, Mountain View, California 94043; 2) Center for Reproductive Sciences; Department of Obstetrics, Gynecology and Reproductive Sciences; Departments of Physiology and Urology; Programs in Human Genetics, Cancer Genetics, and Development and Stem Cell Biology; University of California at San Francisco (UCSF), San Francisco, California 94143.

Human development is dependent upon a cascade of events following fertilization. Unfortunately, knowledge of these critical events in humans is remarkably incomplete. Although hundreds of thousands of human embryos are cultured yearly at infertility centers throughout the world, the vast majority fail to develop in culture or following transfer to the uterus. We used a high sensitivity linear amplification methodology combined with DNA microarray analysis to characterize global patterns of gene expression in single human oocytes and embryos through the first three days of embryonic life. Using real-time RT-PCR, we verified the expression of several genes in healthy oocytes and embryos and then examined expression in embryos with arrested development. Our results provide the first global analysis of human preimplantation transcript profiles, and demonstrate that RNA can be linearly amplified from single oocytes and embryos for analysis using cDNA microarrays. We will summarize the results of our study and discuss future direction for our understanding of molecular mechanisms underlying human pre-implantation development.

A potential role for the interferon response pathway in epithelial cell fate during palate development. *N.K. Rorick*¹, *S.L. Goudy*², *M.I. Malik*³, *J.L. Mihm*³, *C.A. D'Alencon*⁴, *R.A. Cornell*⁴, *B.C. Schutte*^{1,3}. 1) Genetics PhD Program, Univ Iowa, Iowa City, IA; 2) Otolaryngology, Univ Iowa, Iowa City, IA; 3) Pediatrics, Univ Iowa, Iowa City, IA; 4) Anatomy and Cell Biology, Univ Iowa, Iowa City, IA.

Genetic variation in Interferon Regulatory Factor 6 (*IRF6*) causes Mendelian forms and confers risk toward complex forms of cleft lip and palate. *IRF6* is a member of the IRF family of transcription factors. Although best known for mediating the Type I interferon response following a viral infection, several *Irf* transcription factors have been shown to regulate cell proliferation, differentiation and death. These cellular processes are also necessary to determine the cell fate of the epithelium located at the medial edge of the fusing palates in normal development. Previous studies show that stimulation of maternal immunity reduces the frequency of teratogen-induced clefts in mice. We hypothesize that the interferon pathway regulates the cell fate of the medial edge epithelium and that *IRF6* mediates this response. We performed Laser Capture Microdissection and RT-PCR to quantify gene expression in the medial edge epithelium and gel-shift assays to determine DNA binding activity for *Irf6*. We observed that *Irf6* mRNA is expressed in the medial edge epithelium, and is consistent with a role for *Irf6* in determining their cell fate. Also, like other *Irf* transcription factors, we observed that *Irf6* binds the consensus Interferon Stimulatory Response Element, a site that is often located in the promoters of the Type I interferon and interferon-stimulated genes. Additional expression studies show that *Ifna*, but not *Ifnb*, is expressed in the medial edge epithelium. These results are consistent with involvement of part of the interferon pathway in palate development. Finally, whereas several other *Irf* members are expressed in the medial edge epithelium, *Irf5*, the paralog most similar to *Irf6*, is not. In zebrafish, there is partial but not complete overlap of *Irf5* and *Irf6* expression, suggesting that the phenotype caused by mutations in *IRF6* may be restricted to the palate because *IRF5* provides redundant function in other tissues.

Comparative expression patterns of Tbx1, FoxG1b/BF1 and Dlx5 transcription factors during human craniofacial development. *D. Sanlaville, H. Etchevers, M. Clément-Ziza, G. Goudefroye, S. Audollent, E. Detrait, T. Attié-Bitach, M. Vekemans.* Dept Genetics and INSERM U393, Hôpital Necker Enfants Malades, 149 rue de Sèvres, 75743 Paris Cedex 15, France.

We examined the spatiotemporal expression patterns of Tbx1, FoxG1b (also known as BF1) and Dlx5 in normal human embryos obtained from voluntary interruptions of pregnancy, in accordance with ethical guidelines established by French law, using *in situ* hybridization. All three transcription factors are members of gene families with highly conserved DNA-binding domains, for which these and other members have been shown to increase functional specificity through protein-protein interactions. In addition, they all interact genetically with FGFs 8 or 10. In order to see if Tbx1, FoxG1b and/or Dlx5 colocalized during human craniofacial development, we hybridized specific riboprobes to embryonic sections at chosen stages between Carnegie stage (C) 8 and C19. FoxG1b is expressed very strongly at C10 (22 days) in rostral cephalic mesenchyme of mesodermal origin, while Dlx5 is found in the neural folds at the cephalic but not trunk level. During C11-C12 (24-27 days), FoxG1b and Dlx5 are observed in overlapping domains of the prosencephalon. All three factors are expressed in the pharyngeal arches, in the endoderm (FoxG1b, Tbx1) and in neural crest/mesodermal mesenchyme (Tbx1, Dlx5), particularly around the dynamic heart outflow tract connection to the caudal branchial arches, the secondary heart field. At later stages, FoxG1b is maintained in the ventral prosencephalon up to the infundibulum; Dlx5 is seen in the same region but extends caudally into the hypothalamus and distally into the olfactory nerve. Tbx1 and Dlx5 are expressed in similar domains of the otic vesicle and its derivative parts of the inner ear. All three factors also had unique expression domains during embryogenesis. These patterns are consistent with possible physical interaction between Dlx5, Tbx1 and/or FoxG1b during cephalic development, hypotheses that will be tested using functional assays.

Molecular identification of genes and pathways involved in skeletogenesis by EST sequence analysis and functional studies. *S. Schlaubitz*^{1,2}, *C. Stelzer*², *B.U. Zabel*², *B. Lee*¹. 1) Dept. of Mol. and Human Genetics and Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX/ USA; 2) Mol. Genet. Lab., Childrens Hospital, Mainz/ Germany.

An EST-sequencing project was initiated to identify so far undescribed genes in poorly understood pathways related to skeletogenesis. On the basis of 5000 EST clones generated from a human cDNA library of pooled fetal cartilage tissue we obtained sequence data which were compared to the non-redundant Genbank/ EMBL/ DDBJ and dbEST databases using the Ensembl Genome Browser and the EST identification task ESTsweep provided by the HUSAR group in Heidelberg/ Germany as well as the UCSC Genome Bioinformatics browser. An extensive examination of the data obtained by this approach revealed that 77% of EST sequences match to known genes/ mRNAs, 10% to genomic sequences, whereas 9.5% still match to anonymous ESTs (3.5% were contaminations). We scanned these anonymous ESTs for domains by using the SMART browser of EMBL in Heidelberg/ Germany and started to characterize the most interesting clones by Northern Blot analysis with RNA from human, murine and rat cell lines and tissues. Additional RT-PCR and in-situ hybridizations on whole mouse embryos and sections were used as tools to characterize the expression pattern of these genes during development. One of these genes matches a hypothetical protein that shows an interesting expression pattern at different embryonic stages and in northern blot analysis of RNA from different cell lines. Its expression is temporally and spatially restricted. At E13.5, it is expressed in proliferating chondrocytes. By E17.5, it is also strongly expressed in the inner cortex of long bones. Its expression was found in a cell line of murine hypertrophic chondrocytes (MCT, both 32 and 37C), but was absent in murine fibroblasts. Further experiments may help us to understand its involvement in bone pattern formation, growth and development as well as ossification and homeostasis of the skeleton. This gene as well as others within this library may represent candidate genes for skeletal dysplasias as well as for complex disorders (osteoarthritis/ osteoarthrosis and osteoporosis).

Confirmation of a locus on chromosome 5q for a 46 XY Sex Reversal phenotype. *G. Sivapalan, K. Kalidas, Y. Adjei-Gyamfi, S. Sivakumaran, M. Patton, A. Crosby.* Medical Genetics Res, SGHMS, London, United Kingdom.

Genetic sex is determined by inheritance of either an X or Y chromosome. Sex reversal occurs when an individual is genetically one sex but phenotypically the other. There are a number of genes known to play a role within the sex-determining pathway including DAX1 and SOX9. A novel locus on chromosome 5 has also been recently identified in a single family, although the causative gene is as yet unidentified. We investigated a four generation family with multiple individuals affected by XY sex reversal. These individuals are phenotypically female with complete gonadal dysgenesis. Linkage analysis excluded an X-linked mode of inheritance but detected linkage to the recently identified chromosome 5 locus, confirming that a gene for this condition resides in this region. A number of candidate genes are located within this region and are currently under investigation including the gene *FST* which encodes follistatin, a regulator of cell differentiation involved in embryogenesis.

Identification and analysis of Gli3 regulated genes in the murine embryonic limb and face. *C. Wicking, K. Lammerts van Bueren, B. Wainwright, E. McGlinn.* Inst for Molecular Bioscience, University of Queensland, Brisbane, Australia.

Limb and craniofacial defects are amongst the more common features of human dysmorphology syndromes, and evidence suggests the molecular mechanisms governing the development of each of these structures are highly conserved. Using the mouse as a model we have undertaken a microarray screen to identify genes dysregulated in the anterior region of the limb of the polydactylous mutant *extra-toes*. This mutant arose through sporadic deletion of the gene encoding the Gli3 transcription factor, which in the limb is the primary mediator of the secreted morphogen Sonic hedgehog. Mutation of *GLI3* in humans results in a spectrum of developmental disorders including Greig's cephalopolysyndactyly and Pallister-Hall syndrome, both of which involve limb defects. Recent evidence suggests that Gli3 is pivotal to correct anterior-posterior patterning of the limb and is central to specifying digit number and identity. As a result of our microarray screen we have identified a number of both known and novel genes whose expression is altered as a result of *Gli3* loss in the mouse limb. A number of these have been validated by whole mount in situ analysis in the limb, and several show striking expression alterations. Some of these genes also show altered expression domains in the developing face of *Gli3* mutants, providing further support for the molecular conservation of limb and face development. The genes identified in this screen potentially encode missing molecules in the hierarchies governing limb and facial development, and may ultimately prove to contribute to disorders involving these structures.

Inactivation of the Huntingtin disease gene (Hdh) impairs early patterning and primitive streak progression.

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Huntington's Disease, a dominantly inherited neurodegenerative disorder, is caused by a mutation in the huntingtin protein that results in an expanded polyglutamine segment in its N-terminus. Currently, the normal function of huntingtin, a novel 365kDA HEAT domain protein, is unknown. Previous studies have shown that inactivation of the mouse huntingtin gene (Hdh) results in early embryonic death. Expression of huntingtin in the extraembryonic tissues is sufficient to rescue this phenotype in chimeric embryos. To understand the underlying defect in huntingtin deficient embryos, we have examined embryonic patterning and morphogenesis in these embryos. Here, we demonstrate huntingtin is required for node formation and normal primitive streak progression. Additionally, in the absence of huntingtin, posterior restriction of growth factors such as nodal and FGF-8 fails and these factors are ectopically expressed throughout the epiblast. Transient goosecoid expression is also altered, resulting in ectopic goosecoid expression in the posterior epiblast at late streak stages. This failure in posterior restriction does not reflect a loss of anterior/posterior polarity, as normal expression of markers of the anterior epiblast and anterior visceral endoderm (AVE) is observed and the primitive streak and its derivatives are formed in these embryos. Impaired regionalization does not appear to be a result of loss of extraembryonic signal expression, as evidenced by the normal expression of nodal antagonists in the AVE and other extraembryonic signaling molecules in huntingtin deficient embryos. Taken together, these results suggest that huntingtin is essential for normal node formation, primitive streak progression and regionalization in the developing embryo. Support: Hereditary Disease Foundation, NINDS grant NS32765.

Pathophysiology of syndromic combined pituitary hormone deficiency due to a LHX3 defect in light of LHX3 and LHX4 expression during early human development. *M. SOBRIER*¹, *T. ATTIE-BITACH*², *I. NETCHINE*³, *F. ENCHA-RAZAVI*², *M. VEKEMANS*², *S. AMSELEM*¹. 1) Genetique Moleculaire et Physiopathologie,INSERM U468, Hopital Henri Mondor, CRETEIL, France; 2) Handicaps Genetiques de l'Enfant, INSERM U393, Hopital Necker-Enfants malades,PARIS, France; 3) Explorations Fonctionnelles Endocriniennes, Hopital Trousseau, Paris, France.

The pathophysiology of combined pituitary hormone deficiency (CPHD) is just beginning to be elucidated, with mutations in genes encoding transcription factors expressed at different stages of pituitary development. Among them, the two closely related genes, LHX3 and LHX4, are believed to share redundant biological properties. The patients with a LHX3 mutation display a CPHD phenotype, associated with a rigid cervical spine and anteverted shoulders. These latter features, not reported in *Lhx3*^{-/-} and *Lhx4*^{-/-} mice nor in patients with a LHX4 defect, prompted us to study the molecular consequences of a previously identified LHX3 23-bp deletion and to determine the LHX3 and LHX4 expression patterns during early human development. This deletion, which results in the skipping of one coding exon, would lead to a protein with no transcriptional capability. Using in situ hybridization, we show that LHX3 and LHX4 are expressed in the developing pituitary and along the rostro-caudal length of the spinal cord; here, both transcripts are detected in the ventral part giving rise to motoneurons and interneurons. However, whereas LHX3 is expressed at all stages studied, LHX4 expression is transient, and, at 6 weeks of development, is much stronger at the caudal than at the cervical level, thereby suggesting that the extrapituitary anomalies result from a LHX3-dependent neurological defect that is not rescued by the closely related protein LHX4. Overall, these data, which shed light on the pathophysiology of a human developmental disorder, disclose the absence of redundancy between LHX3 and LHX4 during the early stages of human development.

Scd1ab-Xyk: A new allelic asebia model due to a CCC trinucleotide insertion in exon 5 of stearyl-CoA desaturase 1. X. Kong^{1,2,5}, L. Bu^{1,2}, S. Zhou³, M. Jin², J. Sundberg⁴, H. Jiang^{1,2}, M. Qian^{1,2}, Y. Shi^{1,2}, G. Zhao², L. Hu^{1,2}. 1) Health Science Center, sibs., shanghai, shanghai, China; 2) Shanghai Research Center of Biotechnology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200233, Peoples Republic of China; 3) Department of Laboratory Animal Science, Health Science Center, Peking University, Beijing 100083, Peoples Republic of China; 4) The Jackson Laboratory, 600 Main Street, Bar Harbor, Maine 046090-1500, USA; 5) State Key Laboratory of Medical Genomics, Shanghai 200025, People's Republic of China.

Scd1ab-Xyk: A new allelic asebia model due to a CCC trinucleotide insertion in exon 5 of stearyl-CoA desaturase 1
Abstract We found a spontaneous, autosomal, recessive mutant mouse, which arose on the outbred Kunming mice, suffering from skin and hair defects. By haplotype analysis and direct sequencing of PCR products, we describe this mutation as a new allelic asebia one with a naturally occurring mutation in Scd1 (a CCC trinucleotide insertion at nucleotide position 835 in exon 5). This mutation introduces an extra proline residue at position 279 of the Scd1 protein. The mutant mice, originally designated km/km but now assigned Scd1ab-Xyk (hereafter abbreviated abXyk/abXyk), have a similar gross and histologic phenotype as reported in characterized allelic mutations of asebia (Scd1ab, Scd1abJ, Scd1ab2J, and Scd1tm1Ntam). Histological analysis showed they were also characterized by hypoplastic sebaceous glands and abnormal hair follicle. In a cross of Kunming- abXyk/abXyk with ABJ/Le-abJ/abJ mice, all the progeny had the same phenotype indicating these were noncomplimentary and therefore allelic mutations. Comparing with other four allele mutants, this Scd1ab-Xyk carries the mildest change in Scd1 leading to the dysfunction of the Scd1 protein. So this new mutant mouse is a good model not only for studying the human scarring alopecias, which are characterized by hypoplastic sebaceous glands, but also for studying the function and structure of Scd1 protein.

***Tbx1* in the pharyngeal pouch endoderm is required for VCFS/DGS relevant organogenesis.** *J.S. Arnold¹, U. Werling², E.M. Braunstein¹, J. Liao¹, W. Edelmann², B.E. Morrow¹.* 1) Molecular Genetics, Albert Einstein College of Med, Bronx, NY; 2) Cell Biology, Albert Einstein College of Med, Bronx, NY.

Velo-cardio-facial syndrome/DiGeorge syndrome is associated with hemizygous 22q11.2 deletions and characterized by cardiovascular defects, thymic and parathyroid gland hypoplasia or aplasia and craniofacial defects including mild facial dysmorphism, outer and middle ear defects, velopharyngeal insufficiency and submucous cleft palate. Mouse model studies have found *Tbx1*, a gene mapping to the region of synteny to 22q11.2, to be responsible for this syndrome. *Tbx1* is required for embryogenesis and is expressed in multiple interactive tissues during development of the pharyngeal apparatus. Mice heterozygous for a null mutation in *Tbx1* develop mild cardiovascular defects; homozygous *Tbx1* ^{-/-} embryos die at birth with severe congenital defects stemming from abnormal pharyngeal arch (PA) development, including a single outflow tract, thymus and parathyroid aplasia, missing muscles of mastication, absent outer and middle ears and hypoplastic inner ears as well as cleft palate. *Tbx1* is expressed in the pharyngeal pouch endoderm and non-neural crest mesoderm of the PAs and in the otic vesicle. In order to determine the role of *Tbx1* in each interacting tissue during embryonic development, we have conditionally inactivated *Tbx1* in the pharyngeal pouch endoderm and in the otic vesicle epithelium using the Cre/LoxP system. Conditional ablation of *Tbx1* in the *Foxg1* domain causes a severe pharyngeal phenotype including neonatal lethality, thymus and parathyroid aplasia, cleft palate and craniofacial defects. Inactivation of *Tbx1* in the otic vesicle results in absent outer and middle ears and hypoplasia of the inner ear. Overall, *Tbx1* conditional mutants do not differ significantly from the previously described homozygous null mutants. These results underlie the importance of endodermal and otic vesicle driven *Tbx1* expression and suggest that signaling from these structures is critical for proper development of the embryo. We are currently inactivating *Tbx1* in the mesenchyme of PA1-2 to determine its separate role(s) in craniofacial development.

Somitic origin of side population cells in skeletal muscle. *J. Schienda*¹, *S. Jun*¹, *J. Epstein*³, *C. Tabin*², *L. Kunkel*¹, *G. Kardon*². 1) Genomics, Children's Hospital Boston, Harvard Medical School, Boston, MA; 2) Dept of Genetics, Harvard Medical School, Boston, MA; 3) Cardiovascular Division, University of Pennsylvania, Philadelphia, PA.

Within adult skeletal muscle resides a population of cells, side population (SP), which is obtained via FACS analysis of Hoechst dye exclusion. SP cells are a heterogeneous population that contain cells which can radioprotect lethally irradiated mice after intravenous injection and can actively participate in the formation of skeletal myotubes during regeneration. The developmental origin of these cells within the SP population has been controversial. Various researchers have proposed that they are of hematopoietic, vascular or somitic origin. We have examined the potential for somitic origin of the SPs in the chick and mouse. We have labeled chick somitic cells early in development (E2-3) with either replication-defective retroviruses or via quail-chick chimeras (replacing chick somites with quail). Mouse somitic cells were labeled via a Pax3 Mcre x Z/EG transgenic system. SPs were isolated from limb skeletal muscle at ages E14-17 and 5 weeks, respectively, and were found to contain retrovirally-labeled, quail or GFP⁺ cells. When co-cultured with primary myoblasts, these somitic SPs were induced to differentiate into functional myotubes. These results show that the somites are a probable source of adult skeletal muscle-derived SP cells.

Foxc2 is Expressed in Lymphatic Vessels and Other Tissues Associated with Lymphedema-Distichiasis

Syndrome. *S.L. Dagenais*¹, *R.L. Hartsough*¹, *R.P. Erickson*², *M.H. Witte*^{2,3}, *M.G. Butler*¹, *T.W. Glover*^{1,4}. 1) Human Genetics, U. of Michigan, Ann Arbor, MI; 2) Pediatrics, U. of Arizona, Tuscon, AZ; 3) Surgery and Pathology, U. of Arizona; 4) Pediatrics, U. of Michigan.

Mutations in FOXC2 cause hereditary lymphedema-distichiasis (LD) syndrome. LD is an autosomal dominant disorder typically characterized by pubertal lymphedema, and distichiasis, or double row of eyelashes. Less penetrant phenotypes include tetralogy of Fallot, cleft palate, ocular and renal abnormalities. Previous reports showed *Foxc2* expression in mesenchyme of developing heart, kidney and bone. However, its role in the lymphatic system was not characterized, and the basis for lymphatic disease in LD has been unknown. We have conducted a study of *Foxc2* expression during mouse development using immunohistochemistry, and have examined its expression in lymphatics and other tissues associated with LD. We found *Foxc2* expression in lymphatic endothelial cells (LEC) throughout lymphatic development. At E9.5-E10.5, we detected *Foxc2* in cardinal vein endothelial cells, coinciding with the embryonic age and site where lymphatic development is initially detected. We found *Foxc2* in LECs of the jugular lymph sac, and as lymphatic development progresses, it is expressed in the thoracic duct, lymphatic collectors and capillaries. In adult mice, we detected *Foxc2* in lymphatic vessels in the mesentery and skin. We also found that *Foxc2* is expressed embryonically in glomerular podocytes and developing eyelids. We did not detect *Foxc1*, a *Foxc2* paralog, in lymphatics. *Foxc2* nullizygous mice die between E12.5 to a shortly after birth due to heart and skeletal defects. We found that *Foxc2* nullizygous embryos have a lymphatic vasculature; however, lymphatic dilation was seen in an E18.5 embryo. These studies show that *Foxc2* is expressed throughout lymphatic development, suggesting an important role in their development. Also, we can closely correlate expression in various other developing tissues and cell types that exhibit abnormalities in some LD syndrome patients. The exact role of FOXC2 in development of lymphatics and these other tissues should shed light on the pathogenic mechanisms involved in this disorder.

A morpholino based screen for developmental eye defects in zebrafish. *B.R. Bill, A. DeLuca, M.A. Pickart, B.E. Warman, E. Mendenhall, S.C. Ekker, L. Schimmenti.* Depts. of Molecular, Cellular, Developmental Biology and Genetics; Pediatrics and the Arnold and Mabel Center for Transposon Research, University of Minnesota, Minneapolis, MN.

Introduction: We are participating in a large multilaboratory zebrafish screen using reverse genetics to facilitate gene discovery. Our laboratory screens for zebrafish phenotypes that resemble human developmental eye defects, such as microphthalmia, anophthalmia and coloboma. Morpholino oligonucleotides are used to generate loss-of-function phenotypes from sets of genes enriched for predicted secreted proteins or hematopoietic stem cell development. Embryos are screened at 2 and 3 days for gross morphologic defects in eye development specifically optic cup formation, optic fissure closure, pigment localization, and size. **Results:** We are characterizing a phenotype specific to a morpholino against the 5' end of a conserved secreted protein SP2009. By 2 days post fertilization (dpf), the morpholino-injected embryos have larger pupils and microphthalmic eyes compared to wildtype siblings. The penetrance of the phenotype increases with increasing morpholino dose suggesting specificity. The gene product affected by morpholino SP2009 is the zebrafish homolog of the -1-microglobulin bikunin precursor (AMBP) gene. Experiments designed to confirm the specificity of the phenotype to AMBP are underway. We have also discarded a number of phenotypes that appear to be characterized by a disruption of the ventral eye; we believe that this abnormality may largely be due to a common toxicity of the morpholino or a secondary phenotype due to defects in critical developmental processes. Determination of phenotype specificity is an important consideration and possible limitation in the use of morpholino screens for ocular defects. **Conclusion:** The identification of specific eye developmental abnormalities due to defects in conserved genes opens the door to analysis of function and provides candidate genes for human developmental eye defects.

Construction and Screening of Developmentally Staged, Full-Length, Early Embryo Mouse cDNA Libraries for the Identification of Novel Developmental Genes. *D. Konecki, N. Zhou, E. Roessler, M. Muenke.* Medical Genetics Branch, NHGRI/NIH, Bethesda, MD.

The specific interests of our laboratory reside in the study of human genetics disorders (i.e. holoprocencephaly, HPE) resulting from midline and laterality defects occurring early in the development of the human brain. To date mutations in six genes of the more than twenty loci determined to be associated with HPE have been shown to give rise to this disorder. To aid in the identification of additional genes associated with HPE and disorders with similar or related phenotypes, we have constructed and are in the process of screening several early mouse embryo cDNA libraries. These libraries were constructed from gestational time points 7.5 dpc and 9.5 dpc with mRNA isolated from CD-1 mice. SMART technology (Clontech) was implemented in the generation of cDNA cloning material, while TOPO cloning methodologies (Invitrogen) and electroporation were utilized for the production of recombinants. CDNA insert material was sized by agarose gel electrophoresis prior to cloning to be in the size classes of 0.5-1.0 kbp, 1.0-3.0kbp, or 3.0-20 kbp. At the present time, templates from 2016 recombinants have been purified and sequenced using an ABI 3100 Genetic Analyzer. Following editing and trimming to remove vector sequences and long poly(A) tracts, the sequences were submitted for BLAST analysis against the NR Sequence Database established and maintained by the NCBI, resulting in the identification of 64 novel sequences, with no significant sequence homology in the database. Additionally, 52 sequences with polyadenylation signals and poly(A) tracts were detected with >95% homologies only to finished mouse BAC sequences. These and other novel and known genes of interest will be investigated by whole mount in situ hybridization against wild-type (CD-1) and sonic hedgehog (Shh) homozygous and heterozygous mutant embryos of 9.5 dpc gestational age to help identify transcripts involved in the Shh developmental pathway. Other functional screens are currently being assessed to aid in the classification of these previously unknown transcripts.

Cloning and expression of a DAX1 homologue in Zebrafish. *Y. Zhao*¹, *Z. Yang*², *J.K. Phelan*³, *D. Wheeler*⁴, *S. Lin*², *E.R.B. McCabe*^{1,3}. 1) Dept Hum Genet, David Geffen Sch Med at UCLA, LA, CA; 2) Dept Mol, Cell and Dev Bio, UCLA, LA, CA; 3) Dept Pediat, David Geffen Sch Med at UCLA, LA, CA; 4) Human Genome Seq Cen, Baylor Coll Med, Houston, TX.

DAX1 is an unusual orphan nuclear receptor. Mutations in human DAX1 cause X-linked adrenal hypoplasia congenita (AHC), and duplications result in dosage-sensitive-sex reversal. DAX1 is expressed in all regions of the hypothalamic-pituitary-adrenal-gonadal (HPAG) axis. To understand better the roles of DAX1 in the normal HPAG axis development, we use zebrafish as a model organism. We report here the isolation and characterization of a DAX1 homologue in the zebrafish embryo. The isolated DAX1 cDNA clone encodes 264 amino acid residues, including the conserved 3 ligand-binding motif; but the 5 region lacks the unusual repeat motif of the DNA-binding-like domain in mammals. The putative zebrafish DAX1 protein has 50.3% identity and 65.5% similarity with the human DAX1 protein over the 3 region. Stronger identity (65.5%) exists with the tilapia DAX1 over the full-length protein sequence. Genomic sequence analysis indicates that the cloned DAX1 homologue is conserved in gene structure as well: the fish DAX1 is composed of two exons and a single intron, with highly conserved exon-intron boundaries. To explore the expression pattern of this putative DAX1 ortholog, we performed whole mount *in situ* hybridization on zebrafish embryos at various developmental stages. Expression of DAX1 was first detected in the hypothalamus at around 28 hours post fertilization (hpf). Later, a novel expression domain for DAX1 appeared in the fin buds at around 30 hpf. Expression in both locations peaked at 36 hpf. Interestingly, weak and transient expression of this gene was observed in the adrenal at around 31 hpf. Based on the conserved gene structure, the significant protein homology and the shared expression profile, we propose that the cloned DAX1 homolog could be involved in the HPAG axis development in zebrafish. Characterization of the mechanisms of DAX1 action in zebrafish will inform our understanding of the pathogenesis of developmental disorders involving human HPAG, including AHC.

siRNA mediated knockdown of the Survival of Motor Neuron (Smn) protein in cell culture and in transgenic mice. *R. Kothary*^{1,2,3}, *P. Côté*¹, *Y. De Repentigny*¹, *D. Shafey*^{1,2}. 1) Molecular Medicine Program, Ottawa Health Research Inst, Ottawa, ON, Canada; 2) Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Ontario, Canada; 3) Department of Medicine, University of Ottawa, Ottawa, Ontario, Canada.

Spinal muscular atrophy (SMA) is a neurodegenerative disorder characterized by the degeneration of the -motor neurons in the spinal cord. The loss of these neurons causes proximal, symmetrical limb and trunk muscle weakness that progresses to paralysis and ultimately to death. To get a better understanding of the various steps in the pathogenesis of SMA, different knock out and transgenic approaches have been employed to generate suitable mouse models. These mouse models resemble the more severe type of human SMA, but it would be beneficial to generate mice with intermediate and mild phenotypes. In the present study, we describe the use of the short interference RNA (siRNA) approach to selectively reduce *Smn* expression in cell culture and transgenic mice. Double stranded siRNA constructs were targeted to different regions of murine *Smn* and cloned into the psiRNA-hH1neo (from Invivogen) expression vector. This placed the siRNA constructs under the regulation of an RNA polymerase III promoter. The siRNA constructs were transfected into C2C12 muscle cells and *Smn* protein knockdown was achieved. Subsequent to this, we microinjected the siRNA constructs into mouse embryos to derive transgenic founder embryos and mice. Preliminary experiments indicate that the siRNA approach works well in this *in vivo* context. Knockdown of *Smn* protein levels has been observed and we are currently addressing the effect this has on embryonic development particularly as it pertains to the nervous system.

Generating zebrafish models of human muscle disease. *J.R. Guyon¹, S.J. Jun¹, M. Thorne¹, L.S. Steffen¹, E.D. Vogel¹, N. Hsia², G. Ackermann³, Y. Zhou², B. Paw³, L.I. Zon², L.M. Kunkel¹.* 1) Division of Genetics, Childrens Hospital - Boston and the Howard Hughes Medical Institute, Boston, MA; 2) Division of Hematology/Oncology, Childrens Hospital - Boston and the Howard Hughes Medical Institute, Boston, MA; 3) Division of Hematology, Department of Medicine, Brigham & Womens Hospital, Boston, MA.

While many dystrophy associated genes have been identified in mammals, an understanding as to how these genes/proteins contribute to disease pathology is incomplete. To help identify additional proteins involved in the pathogenesis of muscular dystrophy, we have initiated an early pressure screen in zebrafish to genetically isolate additional animals showing symptoms of disease. To date, we have crossed 27 ENU treated males with wildtype females to generate a stock of 2,500 females, each heterozygous for approximately 25 different gene encoding mutations. These females are now being screened using the Early Pressure (EP) technique to generate gynogenetically diploid embryos. Our previous embryonic knockdown experiments have shown that zebrafish embryos lacking either dystrophin or -sarcoglycan are relatively inactive, show decreased muscle organization, show decreased muscle birefringence, and have difficulty inflating their swim bladders. These phenotypic characteristics are now being used to select for muscle mutants with a preference towards those with muscular dystrophy. To date, we have screened 300 of our proposed screen of 600 females and have selected almost 60 potential mutants showing obvious muscle abnormalities or other interesting phenotypes. For example, we have identified potential mutants showing defects in muscle birefringence, muscle organization, left/right patterning, tail structure and pigmentation defects. In order to verify the phenotype, the selected females are now being crossed with wildtype males and the offspring incrossed to see if we can recapitulate the phenotype. The causative mutations for the verified muscle mutants will then be identified using established positional cloning techniques whereas collaborations will be sought to investigate the genetic mechanism for the other interesting mutants.

Developmental defects and impaired short-term synaptic plasticity in Af5q31 mutant mice. *Y. Gu¹, E.J. Weeber^{3,4}, B.A. Antalffy², D. Armstrong², J.D. Sweatt³, D.L. Nelson¹.* 1) Dept Molec & Human Genetics,; 2) Dept of Pathology; 3) Division of Neuroscience, Baylor College of Medicine, Houston, Texas 77030; 4) Current Address: Department of Molecular Physiology and Biophysics, John F. Kennedy Center for Human Development Research, Vanderbilt University Medical Center, Nashville, TN 27232-0615.

Af5q31 is one member of the AF4/FMR2 family, which function as transcription factors. Mutations in FMR2 result in fragile X E mental retardation in humans. There are no reports about simple mutations involved with other members of this family in humans although leukemia has been associated with fusion of MLL gene to AF5q31, AF4 and LAF4, all members of this family except FMR2. To understand the functions of AF5q31, we deleted exon 2, which contains the start codon, in mouse ES cells and developed an Af5q31 mutant mouse model. Several interesting phenotypes have been found in this mutant mouse model, including more than 50% embryonic lethality. This early embryonic lethality is associated with inability of some Af5q31 mutant blastocysts to develop embryos and demonstrates the importance of AF5q31 in the initial embryonic development. This phenotype is not observed with FMR2 and AF4 knockout models; an LAF4 mutant mouse model is not yet reported. The surviving AF5q31 mutant mice exhibited abnormal hair patterns in later life and reduced paired-pulse facilitation in the hippocampus. The abnormal hair patterns result from a shorter catagen stage of hair follicle development cycles; apoptosis plays a role in this stage of hair follicle development. This is the first time demonstrated that functions of AF4/FMR2 family are involved with cell differentiation and apoptosis in vertebrates. Expression of several candidate Af5q31 targets including Sp, TGFbeta and Abl families, has been examined in the Af5q31 mutant mice, and changes were found in several members of Sp family including Sp1 and Zinc 46, a novel member of Sp family, but not in TGFbeta and Abl families.

Anti-sense transcription plays an important role in gene regulation during spermatogenesis. *W.Y. Chan^{1,2,3}, S.M. Wu¹, L. Ruszczyk¹, V. Baxendale¹, E. Law¹, M. Dym³, O.M. Rennert¹.* 1) Laboratory of Clinical Genomics, NICHD/NIH, Bethesda, MD; 2) Departments of Pediatrics, Georgetown University, Washington, DC; 3) Department of Cell Biology, Georgetown University, Washington, DC.

There are numerous examples of anti-sense transcripts in prokaryotes and viruses. On the other hand, only a few examples of anti-sense transcripts in eukaryotes have been reported. A recent computational analysis identified 2,500 pairs of putative sense-antisense transcripts out of 60,770 full-length mouse cDNA (~8%). No example of sense-antisense transcript pairs in eukaryotic germ cell development has been reported. Serial Analysis of Gene Expression (SAGE) was used to profile the expressed genes in mouse type A spermatogonia, pachytene spermatocytes and round spermatids. Sixty two confirmed differentially expressed genes identified in the three types of germ cells with appreciable number of SAGE tags in each were examined for the presence of anti-sense transcripts. Forty two genes showed the presence of anti-sense transcription (65.5%). Of these 42 genes, 35 had overlapping sense-anti-sense transcripts (SAT). Nine of these genes had more than one SAT pairs. Twelve genes had non-overlapping anti-sense bidirectional transcription pairs (NABT). Seven genes had both SAT and NABT. The presence of anti-sense transcripts of selected genes were confirmed by orientation specific RT-PCR and gel electrophoresis. Alignment of the amplified anti-sense transcripts with the genomic sequence of the genes allowed localization of the anti-sense transcripts to exons and introns. 5' and 3' RACE analysis of the anti-sense transcripts and nucleotide sequencing of the cloned fragments were used to examine the size and genomic structure of the anti-sense transcripts of three selected genes, namely, Uba52, Prm2 and Ppp1cc, respectively. Results showed that besides intronic and exonic sequences of the functional gene, anti-sense transcripts could also be derived from pseudogenes. This study showed that anti-sense transcription is a common phenomenon during germ cell development. Anti-sense transcripts can be derived from exons and introns of the functional sense transcript as well as from pseudogenes.

A gene-trap mouse model suggests Nirg1, related to SPG6 involved in spastic paraplegia, functions as a transporter in brain synaptogenesis and renal excretion. J-H. Chai, Q-C. Yu, K. Talbot, E. Pearson, Y. Huang, R.D. Nicholls. University of Pennsylvania, Philadelphia, PA.

A superfamily of *NIPAI/2*-related genes (*NIRGs*) encodes putative 9-transmembrane (TM) domain transporters in species ranging from mammals to bacteria. Human and mouse have six *NIRGs*, with one subfamily including *NIPAI*, mutated in spastic paraplegia type 6. To characterize the other subfamily, we focused on *NIRG1* mapping to human chromosome 1p35-p36 and mouse chromosome 4. Mouse *Nirg1* expression is high in brain, kidney, heart, skeletal muscle, and embryos. We identified a gene-trap (Gt) mouse model with a *geo* insertion in intron 11 of *Nirg1*. Interestingly, a polymorphic 7079-nt IAP element, present in C57BL/6J but absent in 129/Sv, maps ~200-nt from the Gt insertion. Multiplex RT-PCR in heterozygous Gt ES cells (129/129, IAP -/-) vs. brain and liver in heterozygous F₁ mice (B6/129, IAP +/-) suggests that the IAP upregulates *Nirg1* with no effect of the Gt on expression. The Gt allele may be hypomorphic as it expresses all 9-TM domains (exons 2-11) but not the highly conserved C-terminus, which may have a specific function. Intercrossing of Gt F₁ heterozygous (+/-) mice showed non-Mendelian inheritance, with -/- and +/+ homozygotes found at only 20% expected levels in neonates, E7 and E11, with no decrease in litter size. Using X-gal staining, *Nirg1-geo* was detected in -/- and +/- Gt F₂ mice with no signal in +/+. *Nirg1-geo* staining is strong in certain brain regions with a high density of synapses, particularly in mossy fiber terminals of the hippocampal formation and the molecular and granule cell layers of the cerebellum. In kidney cortex and medulla, *Nirg1-geo* is detected in ascending and descending loops of Henle and collecting ducts, in the apical brush border region. Three types of kidney pathology are observed in adult Gt -/- mice: the parietal layer of Bowman's capsule is composed of cuboidal epithelial cells, the glomerulus is also underdeveloped, and many tubules appear dilated, in some instances lacking the regular apical brush border. These data suggest that *Nirg1* is a transporter involved in neuronal synaptic transmission and renal hypertonic urine excretion.

Characterization of Mouse Models for Two Craniosynostosis Syndromes, Apert and Saethre-Chotzen. *Y.L. Wang, R. Xiao, A.J. Iacovelli, D.L. Huso, E.W. Jabs.* Johns Hopkins University, Baltimore, MD.

The neurocranium is normally developed by the process of intramembranous ossification with differentiation of mesenchymal stem cells to osteoblasts without an intermediate cartilaginous stage. Abnormal development can result in craniosynostosis, the premature fusion of cranial sutures. Two autosomal dominant craniosynostosis syndromes are Apert syndrome caused by gain of function mutations of fibroblast growth factor receptor 2 (FGFR2) and Saethre-Chotzen syndrome caused by loss of function of the TWIST gene. To elucidate the pathogenesis of these conditions, transgenic mouse models were designed with mutations analogous to those found in humans. Analysis of the *Fgfr2* S252W/+ mice revealed nascent cartilaginous islands at developing sagittal sutures at E18 and P1. *Sox9* and collagen type II, shown to regulate chondrogenesis, were expressed in the cartilaginous islands. Immunohistochemical and in situ hybridization analyses showed that the expression of Ki67, alkaline phosphatase (ALP), and osteopontin mRNA were increased in the mutant sagittal suture compared with that of wild type. The results indicate that the *Fgfr2* S252W/+ mutation leads to abnormal cell fate determination as mesenchymal cells improperly differentiate into chondrocytes and there is increased proliferation and differentiation of cells in the osteoblastic lineage. Analysis of the *Twist* +/- mice showed increased bone formation and premature ossification in the coronal sutures at E16, E18, and P1. The mutant mice have increased ALP and osteopontin expression, indicating that *Twist* plays a role in suppressing osteoblast differentiation. TUNEL staining revealed increased apoptosis of osteoblast in the mutant coronal suture, signifying that *Twist* may also be involved in the control of osteoblast survival. These studies show that the pathogenesis of craniosynostosis is different for the sagittal and coronal sutures in these two syndromes even though mutations in TWIST and FGFR2 may affect components of the same molecular pathway. Development of the sutures is a complex and delicate balance of several processes involving proliferation, differentiation, and apoptosis.

A role for ATRX in retinal development. *N.G. Bérubé*^{1,2}, *M. Jagla*³, *V. Wallace*³, *D. Picketts*³. 1) Department of Paediatrics and Biochemistry, University of Western Ontario, London, Canada; 2) Child Health Research Institute, London, Canada; 3) Ottawa Health Research Institute, Ottawa, Canada.

ATRX is a component of a large protein complex that exhibits chromatin remodeling activity. ATRX gene mutations in humans cause aberrant methylation of repetitive elements. Affected individuals suffer a multitude of developmental defects that result in mental retardation, delayed motor milestones, as well as urogenital, skeletal, and cardiac abnormalities. The visual and auditory systems are also affected in a subset of cases. The cellular and molecular events that are altered by ATRX mutations and that lead to these developmental defects have not yet been resolved. Using a conditional loss-of-function approach, we show that ATRX is required for the normal development of the murine retina. Loss of ATRX in the retina causes ectopic differentiation of a subset of retinal progenitors accompanied by increased cell death during the embryonic period. Using cell-type specific markers, we observed that the proportion of cell types in the mature retina was perturbed. The number of amacrine cells was greatly reduced and horizontal cells were misplaced within the inner nuclear layer. These results suggest that normal ATRX activity is required for the differentiation and survival of a restricted subset of retinal progenitors during the embryonic period and that loss of ATRX consequently influences the fate and number of different cell types in the mature retina.

Gene-trapping of a tetraspan-like protein disrupts lens fiber cell development in mice. *A. Shiels, J. King, D. Mackay, S. Bassnett.* Ophthalmology/Visual Sciences, Washington Univ Sch Medicine, St Louis, MO.

The human gene for lens intrinsic membrane protein 2 (LIM2) encodes a distant relative of the peripheral myelin protein-22 (PMP22)/epithelial membrane protein (EMP)/claudin (CLDN) sub-family of the tetraspan superfamily of integral membrane proteins. Mutations in LIM2 and its murine counterpart (Lim2) have been linked with autosomal recessive and dominant forms of lens opacities (cataracts), respectively. To gain further insights about loss of Lim2 function we have generated Lim2-deficient mice from a bank of gene-trap embryo stem cells. Genomic PCR amplification and sequencing showed that the gene-trap vector had disrupted intron 3 of Lim2 resulting in a null allele as verified by reverse-transcript (RT)-PCR amplification and sequencing, RNA blotting, immunoblotting and immunofluorescence microscopy. Mice homozygous for the gene-trap (Lim2Gt/Gt) presented with congenital cataracts when the eyes opened on postnatal day 14 (P14). At three weeks of age (P21), Lim2Gt/Gt lenses displayed a discrete opacity centered in the fetal nuclear region. In contrast, heterozygous lenses (Lim2+/Gt) did not develop similar opacities until around 24 weeks of age, however at P21, Lim2+/Gt lenses displayed an optically disturbed nuclear region centered along the optical axis. When crossed with mice transgenic for green fluorescent protein (GFP) expression in all tissues, Lim2Gt/Gt/GFP mouse lens fiber cells underwent normal loss of nuclei and other organelles but failed to develop a characteristic syncytial core region that is believed to facilitate macromolecular diffusion thereby enhancing lens transparency. These results suggest that heterozygous loss of Lim2 is sufficient to induce cataracts in mice and that this functionally enigmatic tetraspan plays a dosage-dependent junctional or adhesive role in establishing the unique refractive architecture of the crystalline lens.

Mediolateral Patterning of the Face; a new mechanism to understand Hypotelorism. *D. Cordero*¹, *M. Tapadia*², *W. Gaffield*³, *J.A. Helms*². 1) Albert Einstein Col Medicine, Bronx, NY; 2) Stanford University School of Medicine, Palo Alto, CA; 3) Western Research Center, US Department of Agriculture, Albany CA.

Recent breakthroughs in molecular and developmental biology have greatly influenced our traditional concepts of how a patient's phenotype correlates with their genotype. Engrained in our clinical acumen is the concept that facial dysmorphologies such as hypotelorism are a consequence of underlying brain anomalies. While the architectural design of the brain certainly plays a role in conditions such as hypotelorism, growing evidence indicates that regions of the brain act as signaling centers, which influence mediolateral and proximodistal patterning of the face. We have investigated the function of one of these signals encoded by Sonic Hedgehog (Shh). Chick embryos exposed to the steroidal alkaloid cyclopamine, which specifically inhibits Shh signaling, develop marked hypotelorism if the treatment occurs prior to Shh induction in the forebrain neuroectoderm. Embryos exposed to cyclopamine at slightly later stages, after Shh induction in the forebrain neuroectoderm, also develop hypotelorism but of a lesser degree. Our analyses revealed an important difference between these two experimental groups, however: in the former case, facial hypotelorism was accompanied by a collapse in mediolateral expansion of the forebrain whereas in the latter case, facial hypotelorism arose independent of brain morphology. Molecular analysis showed that the phenotypic presentation of hypotelorism in the first case is the result of an underlying patterning defect in forebrain neuroectoderm and in the other case was the result of a patterning defect in facial ectoderm. Collectively, our data provides new insights into the molecular mechanisms of how the brain can act as a molecular semaphore, controlling patterning of the face. These studies challenge our traditional views of the etiologies of craniofacial anomalies, and open the door to new strategies to ameliorate or prevent these anomalies.

Williams Syndrome: evidence from atypical deletion links GTFII-I and GTFII-IRD1, with posterior cortical structure and neural functions. *J.R. Korenberg¹, M. Appelbaum², U. Bellugi³, X-N. Chen¹, D. Mills⁴, A. Reiss⁵, F.E. Rose³, L.S. Salandanan¹, A.F. Simon¹, G.M. Yao¹.* 1) Med Gen Inst, Cedars-Sinai, UCLA, Los Angeles, CA; 2) Dept Psych, UCSD, San Diego, CA; 3) Salk Inst Cog Neurosci, San Diego, CA; 4) Emory Univ, Atlanta, GA; 5) Dept Psych Behav Sci, Stanford Univ of Medicine, Stanford, CA.

Williams Syndrome (WS) is a neurodevelopmental disorder caused by a deletion of chromosome 7q11.23. Individuals exhibit striking peaks and valleys in neurocognition that include deficits in visuospatial processing along with relatively preserved language and face processing. These features are associated with distinct variations in neuroanatomy and physiology. In an effort to identify their genetic origins, we have compared 2 atypical cases to WS and to control populations at a highly integrative level, combining studies of molecular structure, volumetric magnetic resonance imaging (MRI), cognition, and event-related potentials (ERPs). We used multicolor FISH with a panel of 45 BACS, PACS and cosmids, PCR of somatic cells hybrids and quantitative Southern blots to identify 80 adults with typical deletions, and 2 adult males with overlapping atypical deletions (one from FKBP6 to CYLN2, and the 2nd from CLDN4 through GTFII-I). Brain structure was determined through MRI studies. Cognition was determined by standard tests and brain electrophysiology by ERPs analyses. Such an approach allowed us to compare the data obtained with the two atypical cases to the distribution for normal and WS subjects. We propose that decreased expression of 2 transcriptional regulators, GTFII-IRD1 and GTFII-I, but not of FZD9, is related to a distinct subset of WS features that includes posterior cerebral variation, visuospatial processing, and abnormal neural activity for face processing. The results of this study begin to define pathways linking gene expression with human cognition.

Increased plasticity of genomic imprinting in brain is due to genetic and epigenetic factors. A.K. Naumova^{1,2,4}, S. Croteau¹, D. Roquis³, M.-C. Charron², D. Frappier⁴, D. Yavin¹, T.J. Hudson^{2,3,4,5}. 1) Department of Obstetrics and Gynecology, McGill University, Montreal, PQ, Canada; 2) Department of Human Genetics, McGill University, Montreal, Quebec, Canada; 3) Genome Quebec and McGill University Innovation Centre, Montreal, Quebec, Canada; 4) The Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada; 5) Department of Medicine, McGill University, Montreal, Quebec, Canada.

Expression of imprinted genes was examined in the brains of mice derived from crosses between C57BL/6 and MOLF/Ei strains. Imprinting of *Igf2r*, *Kcnq1*, *Gtl2*, and *Dlk1* varied among different individuals. As three of these genes are implicated in cell-cell signaling or cell-environment interactions, variation in their imprinting, may influence a wide range of biological processes from cell differentiation to behavior. To elucidate the mechanisms underlying the inter-individual imprinting variation in the brain we focused our effort on the paternally expressed gene *Dlk1* located within the imprinted region of distal chromosome 12. We present evidence that *trans*- and *cis*-acting genetic factors of MOLF/Ei origin facilitate the reactivation of the normally silent maternal allele of *Dlk1*. We mapped one of the *trans*-acting factors to the proximal region of mouse chromosome 7. Reactivation of the maternal allele was also associated with hypermethylation of the maternal copy of the intragenic (IG) DMR, which is the imprinting control region for the *Dlk1* -*Gtl2* domain. Thus, our data suggest that the imprinting status of *Dlk1* in the brain results from interplay between genetic, epigenetic and stochastic factors.

Sexually dimorphic expression in mouse brain of x-linked genes that escape inactivation. *J. Xu, R. Bijlani, A.P. Arnold.* Dept. of Physiological Sci. and Lab of Neuroendocrinology at the Brain Res. Inst., UCLA, Los Angeles, USA.

In mice some X-linked genes escape X-inactivation; most have a Y-linked homologue thought to ensure the dosage balance between the two sexes. To test whether the expression of X escapees is higher in females and X and Y partner genes are expressed in parallel, we examined three such X-Y gene pairs, *Eif2s3x/y*, *Utx/y* and *Jarid1c/d* (aka *Smcx/y*), which have been suggested to be involved in translation initiation, protein-protein interaction, and transcriptional regulation respectively. Using Northern blots, we first confirmed that all three X escapees were expressed at a significantly higher level in the neonatal and adult female brains than in male brains (with the exception of *Jarid1c* in neonates). To determine if the female bias in brain expression is due to the X chromosome dosage effect rather than the sex difference in sex hormones such as estrogens, we used a transgenic mouse model in which there are four genotypes: XX and XY females (with ovaries) and XX and XY males (*Sry* transgene causes testicular development). As expected, XX females and XX males expressed all three genes significantly higher than the two XY genotypes, indicating the XX bias in expression of the three X escapees is a result of sex chromosome complement. Is the sex difference for X escapees compensated by their Y homologues? Previous results suggested differences in expression pattern for *Eif3s3x/y* and *Smcx/y*. However, we found the X and Y partners were expressed in a similar distribution when we examined the regional pattern of expression in adult mouse brain sections with *in situ* hybridization. *Eif2s3x/y* were expressed more highly in brain regions such as hypothalamus, dentate gyrus in hippocampus and habenular nucleus relative to neighboring area. *Utx/y* were found to be highly expressed in brain regions including hippocampus, hypothalamus and cerebellum. The expression pattern of *Jarid1c* and *Jarid1d* was more uniform throughout the brain. A potential sex difference in expression of these X-Y gene pairs suggests a novel mechanism for sexual differentiation of the brain and behavior. (Supported by NS043196 and HD43942.).

Analysis of DNA methyltransferases in Beckwith Wiedemann Syndrome. *E. Algar*^{1,2,3}, *A. Sridhar*². 1) Dept Hematology & Oncology, Royal Children's Hospital, Melbourne, Australia; 2) University of Melbourne, Dept. of Paediatrics, Parkville, Australia; 3) Murdoch Children's Research Institute, Royal Children's Hospital, Melbourne, Australia.

The Beckwith Wiedemann Syndrome (BWS) is an overgrowth disorder in which imprinting mutations affect two centres within 11p15.5, IC1 and IC2. IC1, a 5' region within *H19* is normally methylated only on the paternal chromosome, however 10% of BWS cases acquire maternal methylation at this site. IC2, also known as *KvDMR1/LIT1*, is located 600Kb centromeric to IC1 within an intron of *KCNIQ*, and is normally methylated only on the maternal chromosome. 45% of BWS cases have lost methylation at IC2. Imprinting mutations at IC1 and IC2 are respectively associated with aberrant expression of the 11p15.5 genes *IGF2* and *CDKN1C*.

Mouse models have implicated the DNA methyltransferases *dnmt1* and *dnmt3l* in maternal methylation at the *h19/igf2* locus and indirectly to *cdkn1c* expression in the oocyte and developing embryo. We hypothesize that aberrant expression or function of these genes may therefore underlie the pathogenesis of human BWS. We are screening 100 cases, with either a clinical or molecular diagnosis of BWS, by direct sequencing for evidence of mutations within exonic or regulatory regions of *DNMT1* and *DNMT3L*. To date we have identified four patients with an ARG to CYS change at codon 97 within the DMAP binding domain of *DNMT1*, however this change was also present in unaffected controls. Another patient has a HIS to ARG change at *DNMT1* codon 136. Work is continuing on the analysis of *DNMT1* and *DNMT3L* in patients and controls.

The chromatin remodeling protein Tip60 interacts with FANCD2 in the repair of DNA interstrand crosslinks. *J. Hejna¹, L. Mathewson¹, A. Sobeck², A. Hemphill¹, C. Faulkner¹, M. Matkovic¹, S. Stone², M. Hoatlin^{1,2}, R. Moses¹.* 1) Molec & Med Genetics, Oregon Health & Science University, Portland, OR; 2) Molecular Medicine, Oregon Health & Science University, Portland, OR.

Fanconi anemia (FA) is one of several autosomal recessive diseases characterized by genomic instability and a predisposition to cancer. FA cells are particularly sensitive to DNA interstrand crosslinks (ICLs), manifesting increased cell death and chromosome breaks and radials compared to normal cells. Interactions between various FANC proteins and other proteins implicated in DNA repair have been described, including FANCA and BRCA1; FANCG and BRCA2; BRCA2 and RAD51; and FANCD2 and BRCA2. While the molecular functions of the FANCD2 protein are unknown, its post-translational modification by mono-ubiquitylation in response to treatment with DNA crosslinking agents is required for function of the FA pathway and normal ICL repair. In order to identify additional FANCD2-interacting proteins, we conducted a yeast two-hybrid (Y2H) screen, using FANCD2 as bait. A human fetal brain cDNA library, cloned into the Y2H vector pACT2, was probed with a subclone of FANCD2 encoding amino acids 55-971. Of 2×10^6 colonies screened, one FANCD2-interacting clone, isolated twice independently, was the histone acetyltransferase Tip60, suggesting the involvement of chromatin remodeling in ICL repair. The interaction was confirmed in HeLa cells by colocalization of FANCD2 and Tip60 in nuclear foci, and by co-immunoprecipitation. In addition, siRNA depletion of Tip60 sensitized an immortalized human fibroblast cell line (GM639) to killing by the ICL agent mitomycin C. The results support a model with chromatin remodeling, involving the interaction of Tip60 and FANCD2, as a central requirement for ICL repair.

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Promoter hypermethylation and epi-genetic silencing in retinoblastoma. *C.P. Pang, K.W. Choy, D.S.P. Fan, D.S.C. Lam.* Ophthalmology & Visual Sci, Chinese Univ Hong Kong, Hong Kong, China.

Identification of DNA hypermethylation is an efficient alternative approach for detection of disease associated genes and could have diagnostic applications in cancers. We have shown that promoter hypermethylation that silences expression of the DNA repair gene MGMT is a causative factor of retinoblastoma. To delineate the possible roles of DNA hypermethylation in retinoblastoma, we examined a series of 30 retinoblastoma tissue specimens from sporadic and unrelated patients, and 2 retinoblastoma cell lines (Y79 and WERI-Rb1) by methylation-specific PCR analysis of genes RB1, p14ARF, p15 INK4b, p16INK4a, VHL, MGMT, MLH1, and RASSF1, which were frequently hypermethylated in human cancers. Results of our investigation indicated that aberrant methylation of the MGMT locus was present in a subset (15%) of retinoblastoma tumors, MLH1 in 67% and RASSF1 in 82%. Promoter hypermethylation was associated with null protein expression of all these genes. No hypermethylation on the p14ARF, p15 INK4b, p16INK4a VHL and RB1 genes. was found on tumor DNA. Our study and a report by Harada et al. 2002 both demonstrated that epigenetic silencing as an alternative mechanism by which cancer-related genes are inactivated in retinoblastoma. DNA methylation is associated with the loss of gene function in retinoblastoma. We further propose that epigenetic silencing of multiple cancer related genes may play an important role during development and/or progression of retinoblastoma.

Large-scale chromosome conformation and genome regulation: analysis of the Fragile X locus. *J. Dekker, T. Tabuchi, N. Gheldof.* Dept PGFE, Univ Massachusetts, Worcester, MA.

Large-scale chromosome conformation is emerging as a major regulator of genome activity [1]. Several human diseases, notably cancer and neurological disorders can be caused by changes in chromosome conformation. However, little is known about large-scale chromosome organization and how it modulates genome activity.

We analyzed the Fragile X site to better understand formation of silent chromatin. The Fragile X site contains the FMR1 gene, which is silenced in patients suffering from Fragile X Syndrome, a common form of inherited mental retardation. Silencing is the result of trinucleotide repeat expansion in the first exon, which induces epigenetic changes in the surrounding chromatin. The spatial organization of silent chromatin and the size of the affected region are not known in detail. We analyzed large-scale chromosome conformation of the Fragile X site using 3C technology [2, 3]. 3C (Chromosome Conformation Capture), a relatively new technology that we originally developed, is used to detect interactions between genomic loci revealing the spatial organization of a genomic region. We find that local trinucleotide repeat expansion has large-scale effects on chromatin conformation. A region of at least 250 kb displays increased compaction. Interestingly, the level of compaction fluctuates throughout this region, suggesting the presence of sequence elements that play a role in spreading of silent chromatin.

We are part of the new ENCODE consortium initiated by the National Human Genome Research Institute (<http://www.genome.gov/ENCODE/>). The goal of this consortium is to identify all functional elements in the human genome. We use 3C technology to identify interacting elements involved in transcription and chromosome morphogenesis and have devised a general strategy for 3D structural annotation of human genomic regions.

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Maternal duplication of chromosome 11p15 in a young girl with growth retardation and dysmorphic features.

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Paternal duplication of the short arm of chromosome 11 is a well-described cause of Beckwith-Wiedemann Syndrome (BWS). The chromosomal region of 11p15 harbours many imprinted genes. Dysregulation of the expression of imprinted growth-related genes found in this region are thought to be responsible for the overgrowth phenotype and risk of malignancy seen in individuals with BWS. We describe a 17 month-old girl with growth retardation, dysmorphic features who has three copies of 11p15, two of which are of maternal in origin. Our patient was born to a healthy 31 year-old mother at 36 weeks gestation. Symmetrical intra-uterine growth restriction was noted on prenatal ultrasound. Her birth weight was 1.84 kg(3rd percentile). Physical features included a prominent forehead, hypertelorism, low-set ears, up-slanting palpebral fissures, and 5th finger clinodactyly. She was found to have an ectopic non-functioning left kidney, right nephrocalcinosis and a small spleen. In the neonatal period, she had periodic breathing and required oxygen therapy. She has failure to thrive and is G-tube fed. Her hearing is decreased on the left and she has strabismus. Initial chromosome analysis revealed a karyotype of 46,XX,add (10)(q26) at 500-550 band resolution. Further characterization using fluorescent in situ hybridization showed a karyotype of 46,XX,add(10)(q26).ish der(10) t(10;11)(q26.3;p15.1)(WCP 11+, tel 10q-, tel 11p+). BAC probes for the regions 10q26.3 and 11p15.2 showed two and three signals respectively. Microsatellite repeat analysis identified the duplicated material of chromosome 11p to be of maternal origin. There are three other published cases of maternal duplication of chromosome 11p15 and all have growth retardation. Our findings are consistent with the proposed model that growth-promoting genes on chromosome 11 are paternally expressed and growth-suppressing genes are maternally expressed. A dysregulation of the maternally expressed growth-suppressing genes such as H19 and p57^{KIP2} may to responsible for the growth retardation seen in patients with maternal duplication 11p15.

Disruption of epigenetic regulatory elements and chromosomal aberrations in patients with Beckwith-Wiedemann syndrome. *A.C. Smith^{1,2}, J.A. Squire², C. Shuman¹, R. Weksberg¹*. 1) Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Institute of Medical Sciences, Dept. Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada.

Beckwith-Wiedemann syndrome (BWS) is characterized by somatic overgrowth, macroglossia, omphalocele, and tumor predisposition. It is associated with dysregulation of gene expression of an imprinted gene cluster on chromosome band 11p15. The 11p15 region is divided into two domains each controlled by an imprinting centre for each domain. Imprinting centres can be differentially methylated and associated with anti-sense transcripts that can regulate the expression of neighboring genes in cis over large distances up to one megabase. The KCNQ1 differentially methylated region (DMR2) is found within intron 10 of KCNQ1 in Domain 2. DMR2 also contains the promoter for KCNQ1OT1, a paternally expressed, untranslated anti-sense transcript, which is believed to suppress the expression of nearby genes on the paternal chromosome e.g. KCNQ1 and CDKN1C. About 50% of patients with BWS have a loss of methylation at DMR2 and biallelic expression of KCNQ1OT1. A variety of other genetic and epigenetic molecular alterations comprise the other 50%.

We have 7 translocation or inversion patients (3 with BWS, 4 without) that have breakpoints within 500kb of DMR2. We expected that translocations and inversions associated with BWS would disrupt the imprinting centre in Domain 2; however, we found that BWS patients with translocations have normal DMR2 methylation and expression of KCNQ1OT1. Our data suggest that there are as yet unidentified mechanisms that can cause BWS and its associated tumors. Thus, we hypothesize that physical disruption of the region and regulatory signals other than DMR2 methylation and KCNQ1OT1 transcription in Domain 2 can alter imprinted gene expression in cis. In this regard, we are currently assessing allele specific expression of the imprinted genes in Domain 2. The results of this investigation will further elucidate the mechanisms of epigenetic control on chromosomal region 11p15.

Genomic analysis of human neocentromeres. *A.L. Alonso¹, M. Paziienza¹, F. Cheung¹, J. Tepperberg³, J. Engelen², P. Papenhausen³, M. Ventura⁴, M. Rocchi⁴, P. Warburton¹.* 1) Mount Sinai, New York, NY; 2) Academic Hopspital, Maastricht; 3) LabCorp, NC; 4) University of Bari.

Neocentromeres are new human centromeres that are devoid of alpha satellite DNA and have arisen ectopically in chromosome arms, permitting their genomic position to be precisely mapped onto the underlying DNA sequence. In order to provide insight into epigenetic mechanisms for neocentromere formation, we have performed genomic analysis of multiple neocentromeres from bands 8q21, 13q21, and 13q32. Chromosome 13q represents a neocentromere hotspot, containing 13 (18.5%) of the 70 cases thus far described, with 4 cases in 13q21 and seven cases in 13q32. To position 13q neocentromeres, we used ChIP on a CHIP assays with antibodies to CENP-A, the centromere-specific histone H3, and screened contiguous genomic BAC microarrays spanning 13q21 (11.4Mbp) and 13q32 (14Mbp). Human 13q21 neocentromeres may represent the reemergence of ancestral centromeres, since centromeres in several old world monkeys correspond to human chromosome band 13q21. The CENP-A binding domain of a 13q21 neocentromere was localized to a 280kb domain, which was several Mbp distal to the old world monkey centromeres. An ~11mb 13q21 neocentric ring chromosome also maps distal to the old world monkey centromeres. The CENP-A chromatin of three neocentromeres that all cytologically localized to band 13q32 was found to form on distinct genomic sequences of 130kb, 215kb and 275kb within a 7mb region. CENP-C, a constitutive centromere protein, also colocalized to the 130kb CENP-A domain. A unique neodicentric chromosome 8, with an inactivated endogenous centromere and a neocentromere in band 8q21, was found fortuitously in a phenotypically normal male. 2 similar recently observed neodicentric chromosomes 3 and 4 represent an emerging class of novel chromosome variants. Molecular cytogenetic comparison of the 8q21 neodicentric chromosome to a neocentric ring 8q21 chromosome showed that they do not share a common neocentromere site. Overall, these studies show a remarkable plasticity in the genomic position of neocentromeres, and support a sequence-independent epigenetic model for neocentromere formation.

Epigenetic Variegation in Long DNA Palindromes. *A.G. Cote^{1,2}, S.M. Lewis^{1,2}*. 1) Genetics and Genomic Biology, Hospital for Sick Children, Toronto, Canada; 2) Department of Molecular and Medical Genetics, University of Toronto, Toronto, Canada.

DNA palindromes are perfect inverted repeats with two-fold rotational symmetry and have the ability to form hairpins and cruciform structures by intrastrand base-pairing. These arrangements are unstable in the genome and are frequently rearranged. It is postulated that they are substrates for chromosomal deletions, insertions, gene amplification, and translocations, but the mechanism responsible for palindrome instability is unknown nor have any factors been identified that affect this process. The Line 78 mouse bears a 15.4kb perfect palindromic transgene that rearranges at a high rate in somatic cells. We report that the degree of DNA methylation of the Line 78 palindrome can vary between completely isogenic cell lines in culture. Variegating patterns of methylation do not correlate with the acquisition of a central palindrome modification event, where the differences in methylation are observed in a number of cell lines that all harbour an intact, unmodified palindrome. Cell lines carrying a fully methylated or an undermethylated Line 78 palindrome will be analyzed to determine if there exists a relationship between DNA methylation and instability of long palindromes. The Line 78 palindrome's rate of rearrangement will be assessed for each cell line to determine if variations in the palindrome's pattern of DNA methylation are associated with significant differences in the rate of rearrangement.

A fraction of mouse minor satellite DNA is favoured for centromere function on a mini-chromosome in human, mouse and chicken cells. *M.H. Shen¹, K. Zeng¹, J.I. de las Heras¹, A. Ross¹, H. Cooke¹, J. Yang².* 1) Chromosome Biology, MRC Human Genetics Unit, Edinburgh, Scotland, United Kingdom; 2) Institute for Stem Cell Research, University of Edinburgh, West Mains Road, Scotland, Edinburgh EH9 3JQ, UK.

Centromeres are required for faithful segregation of chromosomes in cell division. It is not clear how the centromere sites are specified on chromosomes in vertebrates. We have previously introduced a mini-chromosome, named ST1, into a variety of cell lines including human HT1080, mouse LA9 and chicken DT40. This mini-chromosome, segregating faithfully in these cells, contains mouse minor and major, and human Y alphoid satellite repeats. In this report, we examine the requirement of DNA sequences for centromere function of ST1. After determining the organisation of the satellite repeats, we investigated the centromere location on this mini-chromosome by combined analysis of immunocytochemistry and FISH. Centromeric proteins were consistently co-localised with the minor satellite repeats and the CENP-B boxes embedded in these repeats in all the three cell lines. When chromatin fibres were highly stretched, centromeric proteins were only seen on a small portion of the minor satellite repeats. These results demonstrate that a fraction of the minor satellite repeats is fully competent in centromere function not only in mouse but also in human and chicken cells while the human Y alphoid DNA on this mini-chromosome is disfavoured for centromere activity. These findings suggest a sequence favour model for possible mechanisms specifying a centromere site upon the establishment of hybrid cell lines.

Heterogeneous distribution of methylated histone H3 and macroH2A in heterochromatin of the human inactive X chromosome. *C.M. Valley, B. Chadwick, H.F. Willard.* Inst Genome Sciences/Policy, Duke University, Durham, NC.

In female mammalian cells, one X chromosome is transcriptionally silenced to equalize X-linked gene expression between the sexes. Several features distinguish the inactive X chromosome (Xi) from the active X (Xa), including late replication in S-phase, trimethylation of lysine 9 on histone H3 (H3TrimK9), and association with both XIST RNA and the histone variant macroH2A. To explore the cytological, molecular and genomic distributions of these features, we have used immunocytochemistry, analysis of late replication, and chromatin immunoprecipitation (ChIP) to detect both H3TrimK9 and macroH2A in human primary cell lines. Cytologically, macroH2A and H3TrimK9 appear enriched on the Xi, but occupy separate territories at interphase and metaphase in alternating bands that are both spatially and temporally distinct. The latest replicating regions on the Xi are associated with H3TrimK9, but not macroH2A, while regions replicating in mid-S phase are associated with macroH2A and with XIST RNA, but not H3TrimK9. To examine their composition at a higher resolution and to better understand their relationship on the Xi, we next determined the enrichment of macroH2A and H3TrimK9 on the Xi. Over 120 informative X-linked SNPs were identified in a female cell line and examined by ChIP in combination with a quantitative assay to discriminate the Xi and Xa alleles. The resulting profile revealed a ~2- to >10-fold enrichment of either macroH2A or H3TrimK9 on the Xi for the majority of SNPs and correlated with their location in either a macroH2A- or a H3TrimK9-associated band, confirming the cytological data. However, a few SNPs gave intermediate or reverse patterns, suggesting an additional level of heterogeneity at the molecular level. We conclude that the composition of facultative heterochromatin on the Xi is more complex and heterogeneous than previously believed. Extension of this analysis to additional SNPs at high density and correlation with both the underlying DNA sequence and the Xi expression profile should provide insights into the chromosomal and genomic basis for X inactivation.

A role for genomic imprinting in male sexual orientation. *S. Bocklandt*¹, *C.E. Roselli*², *S. Horvath*³, *M.G. DuPree*³, *E. Vilain*¹, *D.H. Hamer*³. 1) Dept Human Genetics, David Geffen School of Medicine, UCLA, Los Angeles, CA; 2) Department of Physiology and Pharmacology, OHSU, Portland, OR; 3) Laboratory of Biochemistry, National Cancer Institute, NIH, Bethesda, MD.

Genomic imprinting of X-chromosome genes could lead to sex-specific gene expression, and is therefore a candidate mechanism for the regulation of sex specific traits, such as sexual orientation. Since X-inactivation and genomic imprinting share several molecular mechanisms, we measured X-inactivation in mothers of gay men to test the role of imprinted X genes in sexual orientation. We analyzed X chromosome inactivation in 96 mothers of gay men and 105 control women matched for age and ethnicity. Skewing was assayed in white blood cell DNA at the androgen receptor gene and at the fragile X locus. We found a significant difference ($p=0.004$) in X chromosome skewing between the two groups. The mothers of gay men showed a bimodal distribution in which 14% displayed extreme skewing (more than 90% inactivation of one chromosome). The control women showed a normal distribution in which less than 3% had extreme skewing. Analysis of mouthwash samples showed a high correlation between skewing in blood and buccal cells. Daughters of mothers with extreme skewing were analyzed, and only 1/24 was skewed, indicating that it is not caused by an X chromosome mutation. In order to identify the exact mechanisms of genomic imprinting in sexual orientation, we undertook two complimentary approaches, based on linkage and expression profiling. A full genome linkage scan was performed on 146 gay brother pairs. Significant linkage to 10q26 was found on the maternal allele only, indicating a maternally expressed, paternally silenced, imprinted gene involved in sexual orientation. Micro array expression analysis of cell lines from gay and straight men and their families was performed. In addition, cross species micro array hybridization was performed on the anterior hypothalamus and amygdala of male oriented and female oriented rams. Candidate genes derived from a combination of human and sheep data have been identified. Together, these data suggest that genomic imprinting influences male sexual orientation in mammals.

Methylation-specific MLPA: a novel method for the methylation status of multiple CpG sites of tumor-suppressor genes. *A.O.H. Nygren^{1,2}, S. Guerrero Caballero^{1,2}, H.M. Duarte¹, R.N.C.P. Vijzelaar¹, G. Pals², J.P. Schouten¹, A. Errami¹.* 1) MRC-Holland, Amsterdam, Netherlands; 2) Department of Clinical Genetics, VU Medical Center, Amsterdam, Netherlands.

We have developed a novel method, methylation specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA), to detect CpG methylation and copy number of up to 26 genes in a single reaction. This method combines digestion of genomic DNA with methylation-sensitive restriction enzymes and MLPA. The methylation sensitive probes have a design similar to that of ordinary MLPA probes except that the sequence detected by the MS-MLPA probes contains a recognition sequence for the methylation sensitive restriction enzymes HpaII or HhaI. Upon digestion with one of these enzymes, probes of which the recognition sequence is unmethylated will not generate a signal. In contrast, a probe signal will be detected if the site is methylated. This method is very easy to use and requires only 20 ng of DNA. Furthermore, it has the advantage to study simultaneously several genes in contrast to existing methods that only allows the study of one gene or locus at a time. In addition, we show that this technique can be applied successfully to establish the methylation status in tumor DNA obtained from paraffin-embedded tissue samples. To prove that MS-MLPA is a powerful technique for the detection of methylation changes of promoter regions, we used MS-MLPA in DNA samples of patients with Prader-Willy and Angelman syndrome.

Epigenetic and molecular genetic findings in Russian Rett syndrome cohort: linking the epigenetics to MECP2 gene mutations. *S.G. Vorsanova¹, I.Y. Iourov², V.Y. Ulas¹, L. Villard³, L. Gianti⁴, M.-L. Giovanucci-Uzielli⁴, Y.B. Yurov².* 1) Molecular Cytogenetics, Institute of Pediatrics, Moscow, Russian Federation; 2) National Center of Mental Health RAMS, Moscow, Russia; 3) INSERM U491, Faculte de Medecine, Marseille, France; 4) University of Florence, Florence, Italy.

We have screened 39 females and 1 male with Rett syndrome (RTT) for the MECP2 mutations and 84.6%; (33 from 39) of them were found to have a MECP2 mutation. The boy studied had a R270X mutation. We have studied X-inactivation in these 39 females. The incidence of skewed X-inactivation was 41% (16 from 39). This value is significantly higher than for control group (6.5%, $p < 0.001$). We have also analyzed X chromosome inactivation patterns in another 31 RTT patients not screened for MECP2 mutation. Skewed X-inactivation was determined in 10 (31.1%) RTT girls being significantly different from control group and insignificantly different from RTT females screened for MECP2. Therefore the total incidence of skewed X-inactivation in RTT females studied is estimated as 37% (26 from 70). We have also carried out the study of preferentially inactivated chromosome X determination by assessing the X-inactivation in RTT females and their mothers. About 33% had a maternal X chromosome to be preferentially inactivated. This analysis allowed us to determine genotype-phenotype correlations. The more severe forms of the disease were observed in cases with preferentially inactivated maternal X chromosome and milder forms were associated with preferentially inactivated paternal X chromosome. We have proposed two hypotheses explaining skewed X-inactivation higher incidence in RTT. Firstly, the unstable transcription of some X-linked genes could be caused by MECP2 mutations and lead to X-inactivation skewing. Secondly, MeCP2 is known to be involved in repression of LINE-1 expression. The abnormal expression of these elements located on chromosome X may also lead to skewed X-inactivation. These assumptions should be carefully tested. However the data obtained is clearly indicating the relation of this epigenetic phenomenon and MECP2 mutations.

X-chromosome inactivation and premenopausal breast cancer. *J.P. Struewing¹, M.A. Pineda¹, M.E. Sherman², L.A. Brinton², J.M. Lissowska³, M. Garcia-Closas².* 1) Lab of Population Genetics, and; 2) Hormonal and Reproductive Epidemiology Branch, National Cancer Institute, Bethesda, MD; 3) Division of Cancer Epidemiology and Prevention, Cancer Center and M. Sklodowska-Curie Institute of Oncology, Warsaw, Poland.

Each cell of a female fetus randomly inactivates one X-chromosome during embryogenesis, resulting in a population of cells that express roughly equal quantities of transcripts from maternally and paternally derived X-chromosomes. Skewed X-chromosome inactivation is present if most or all of the transcripts are derived from only one of the parental X-chromosomes. A trinucleotide-repeat in the androgen receptor gene (*AR*) on the X-chromosome is highly polymorphic and methylation at two CpG sites near the repeat correlates with silencing. This allows one to assess skewed inactivation by comparing relative peak heights for the polymorphism in DNA digested with a methylation-specific restriction enzyme and undigested DNA. Skewed X-inactivation in lymphocytes has been associated with increased risk of ovarian cancer in a US study and with early-onset breast cancer in a Norwegian study. The recent observation of the involvement of the BRCA1 protein in normal X-chromosome inactivation further highlights the possible involvement of skewed X-chromosome inactivation in breast cancer. We are assessing the association between skewed X-chromosome inactivation and breast cancer by testing lymphocyte DNA of a subset of 585 subjects enrolled in a population-based case-control study from Warsaw and Lodz, Poland using the *AR* assay. Subjects include 305 premenopausal invasive breast cancer patients (mean age 46 years, 80% below age 50) and 280 age-matched controls. Preliminarily, among 362 heterozygous women tested to date, 27.4% (n=46) of cases and 26.8% (n=52) of controls showed skewed X-chromosome inactivation. Using a more stringent cut-point for categorizing a sample as skewed, cases were slightly less likely to show skewing (5.8%) than were controls (7.6%). Based on preliminary, unadjusted analyses, there appears to be no difference in skewed X-chromosome inactivation between pre-menopausal breast cancer cases and controls.

A novel deletion in the Prader-Willi imprinting center narrows the critical region. *H. Dong, M. Whitney, P. Kuipers, D. Driscoll.* Dept. of Pediatrics and Molecular Genetics & Microbiology, University of Florida, Gainesville, FL.

Angelman (AS) and Prader-Willi (PWS) syndromes are complex neurobehavioral conditions involving the loss of expression of imprinted genes in a 2 Mb region on human chromosome 15q11-q13. AS is due to loss of maternal-only expressed genes and PWS is due to the loss of paternal-only expressed genes from this region. The 2 Mb imprinted domain is under the control of a bipartite imprinting center (IC) comprising two distinct regulatory regions. Several unique PWS microdeletion families have narrowed the critical region for PWS to a 4.3 kb area including the SNURF-SNRPN promoter, CpG island and exon/intron 1. The AS smallest region of overlap (SRO) has been narrowed by patients with microdeletions to an 880 bp sequence 35 kb upstream of the PWS-SRO. The PWS-SRO has a strong DNase I hypersensitive site specific for the paternal allele and 6 evolutionary conserved (human-mouse) sequences that are potential transcription-factor binding sites.

In this study we describe a unique PWS patient with an IC deletion that further narrows the distal boundary of the IC critical region. Multiple restriction enzyme digests and Southern blots revealed a 24 kb deletion. PCR primers were designed to allow us to sequence the proximal and distal breakpoints, which revealed that the distal breakpoint is 385 bp into intron 1 of the SNURF-SNRPN gene. DNA methylation analysis of MKRN, PW71 and SNURF-SNRPN demonstrated a maternal uniparental pattern. Expression analysis showed loss of expression of MKRN3, NDN, SNURF-SNRPN and IPW that is consistent with PWS. We are currently in the process of evaluating histone modifications in the AS/PWS region in this unique PWS-IC deletion patient to correlate with our expression and DNA methylation results.

Our findings are important since they narrow the PWS-SRO to 4.1 kb and delineate the exact sequence of the proximal and distal breakpoints of this IC microdeletion. These data should provide a foundation for a better understanding of the genesis of IC microdeletions and the mechanisms involved in the imprinting process in the AS/PWS region.

Association of the Polymorphism IGF2/ApaI with birth weight and body mass index. *M.V.M. Gomes¹, M.R. Soares^{1,2}, C.R. Marcondes¹, A. Pasqualim-Neto², R.B. Lobo¹, E.S. Ramos¹.* 1) Department of Genetics, FMRP-USP, Ribeirão Preto, São Paulo, Brazil; 2) University Center Barão de Mauá, Ribeirão Preto, São Paulo Brazil.

Insulin-like growth factor II (IGF-II) has a high degree of sequence homology with insulin and plays a key role in mammalian growth, influencing fetal cell division and differentiation and possibly metabolic regulation. The gene IGF2 is subject to parental imprinting and only expressed from the paternal allele. Studies using pigs have implicated the *Igf2* gene in fat deposition. In humans the ApaI polymorphism at the exon 9 of the IGF2 has large been associated with alteration involving weight and obesity predisposition. In the present study we examined the association of IGF2/ApaI genotype with body index mass (BMI) in healthy volunteers (95 men and 199 women; 18-30y) and correlated the results with the birth weight (BW) of the subjects. Blood sample were obtained for DNA extraction and RFLP-PCR. Normal distribution for BMI and BW was showed by Kolmogorov-Smirnov normality test. The highest value of BW in the genotype groups (AA=3.87kg, AG=4.68kg and GG=4.95kg) could indicate a gradual increasing in the weight related to the presence of allele G. The genotype was statistically significant (p.05) for BW but the effect of the sex did not (p.23). The existence of other sources of variations in BW, which were not controlled in this experiments, was indicated by the low R-square of the model (R²=0.03). There is no evidence of statistically significant differences between the least-square means of each genotype, for both BW and BMI (AA: BW=3.21 and BMI=22.66; AG: BW=3.30 and BMI=23.06; GG: BW=3.42 and BMI=23.76), by the Tukeys pos hoc tests (=0.05). No statistically significant correlation (p.05) were observed between BW and BMI in each genotype group. Nevertheless a statistically significant correlation (p.023) and equal .33 was observed between BW and BMI in GG subjects whose BW was higher than 3.5 kg (n=47). Although the IGF2 genotype was not significantly associated with BMI and BW, there was a non-significant trend for babies with the GG genotype to be heavier than those with the AA genotype.

Quantitative analysis of mosaic DNA methylation in patients with Angelman syndrome. *H. Nazlican¹, M. Zeschnick¹, U. Claussen², S. Michel², G. Gillessen-Kaesbach¹, S. Gross¹, C. Lich¹, S. Boehringer¹, B. Horsthemke¹, K. Buiting¹.* 1) Universitätsklinikum Essen, Institut für Humangenetik, Essen, Germany; 2) Institut für Humangenetik, Universität Jena, Germany.

In a small group of patients with Angelman syndrome (AS) the disease is due to an imprinting defect. Among 92 such patients, 11 patients (12%) were found to have an imprinting center (IC) mutation, whereas in 81 patients (88%) no mutation could be detected. The presence of a faint maternal band detected by methylation specific PCR analysis of the *SNRPN* locus in one third of non IC mutation patients suggests that these patients are mosaic for an imprinting defect that occurred after fertilization. In one patient studied, somatic mosaicism was proven by methylation analysis of cloned fibroblasts. X inactivation studies of these clones suggest that the imprinting defect occurred before the blastocyst stage, when the maternal genome undergoes global demethylation. To quantify the degree of methylation at the *SNRPN* locus, we developed a novel quantitative real time PCR-based assay (TaqMan) based on bisulfite treated DNA. We used two primers that bind to DNA sequences unaffected by differential DNA methylation and two minor groove binding probes specific for the unmethylated and the methylated allele, respectively. A standard curve was constructed by mixing varying amounts of DNA from a patient with Prader Willi syndrome with uniparental disomy (two methylated gene copies) and a patient with AS with a typical deletion (one unmethylated gene copy). In 24 patients tested, the percentage of normally methylated cells ranged from 1-32%. A genotype-phenotype correlation suggests that patients with a higher percentage of normally methylated cells tend to have milder clinical symptoms than patients with a lower percentage.

ANALYSIS OF EPIMUTATION IN THE 15q11-13 IMPRINTED DOMAIN IN AUTISM. *M. Shinawi*¹, *R. Wagle*¹, *T. Sahoo*¹, *R.J. Schroer*², *R. Stevenson*², *A.L. Beaudet*¹. 1) Dept Molec Human Genetics, Baylor Col Medicine, Houston, TX 77030; 2) Greenwood Genetic Center, South Carolina 29646, USA.

The genome wide linkage studies have failed so far to identify major autism-related loci suggesting that many loci might contribute to the etiology of autism. We have proposed that there may be an epigenetic component to the etiology of autism. The maternally inherited duplication of 15q11-q13 among autistic patients indicates gene-dosage and parent-of-origin effects in this domain. In previous studies, we showed increased sharing for the paternal haplotypes at *D15S817*. We hypothesized that epigenetic and genetic factors may cause autism by altering the expression of principal imprinted genes in 15q11-q13 and that a pre-zygotic or post-zygotic imprinting defect (imprint-switch failure) in the imprinting center of the domain 15q11-q13 could be the cause of some of the cases of autism. To determine the grandparental origin of the imprinted PWS/AS domain, we genotyped fathers and mothers from the AGRE, NIMH, and South Carolina Autism Project (SCAP) collections by means of analysis for the *HpaII/MspI* RFLP in intron 1 of *SNRPN* and analyzed the informative families by a combined RFLP/ methylation-specific PCR analysis of the *SNRPN* locus. The AGRE and NIMH samples were mostly sib pairs and the SCAP samples mostly isolated cases. By analyzing 172 autistic individuals, there was no statistically significant difference in the overall sample for whether the 15q11-q13 domain was inherited from the grandmother vs. grandfather on the paternal side or for the grandmother vs. grandfather on the maternal side. There was a borderline significance favoring inheritance from the paternal grandmother ($P = 0.05$) and from the maternal grandmother ($P = 0.01$) in the SCAP group, but it is unlikely that both would be significant, and neither was found for the NIMH or AGRE groups. Considering the inherently greater risk for a chromosome that must switch imprint vs. not switch, it may be appropriate to further evaluate the risk of imprint defects inherited from the paternal grandmother in sporadic cases of autism.

Molecular mechanism of reciprocal imprinting of the mouse *Grb10* gene during brain development. Y.

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The mouse *Grb10* is an imprinted gene with tissue-specific and promoter-specific expression. In most tissues, *Grb10* is expressed exclusively from the major promoter of the maternal allele, whereas in the brain it is expressed predominantly from the brain-specific promoter of the paternal allele. Such reciprocally imprinted expression in the brain and other tissues is thought to depend on the alternative usage of promoters and insulator activity on the differentially methylated region (DMR). To investigate the mechanisms of brain-specific *Grb10* imprinting, we focused on epigenetic modifications in neuronal cells in the brain. *Grb10* expression, DNA methylation and histone modifications were separately analyzed in neurons, glial cells and fibroblasts by using primary cortical cell cultures. RT-PCR analysis demonstrated that imprinted paternal expression of *Grb10* in the brain was promoter-specific for neurons but not for glial cells and fibroblasts, which showed reciprocally maternal expression same as in other tissues. Methylation analysis of putative CpG islands revealed that the DMR in the brain-specific promoter region was detected not only in neurons, but in glial cells and fibroblasts, while other CpG islands including in the major promoter were not methylated in the cultured cells. Histone modifications in DMR was analyzed by chromatin immunoprecipitation(ChIP) assay. Neurons were associated with paternal-allele-specific high acetylation of H3 and H4 and dimethylation of K4 and preferentially maternal-allele-dominant dimethylation of K9, although hypoacetylation of H3 and H4 was detected in glial cells and fibroblasts. These data indicate that reciprocally imprinted *Grb10* expression in the brain depends on neuron-specific histone modifications related to the lineage determination of neural stem cells, but not to DNA methylation.

Loss of AGG Interruptions from Gray-Zone FRAXA (CGG)_n Tracts: Mapping Tract Length Changes & Deletion Breakpoints. *K. Nichol Edamura*¹, *C.E. Pearson*^{1,2}. 1) Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Department of Molecular & Medical Genetics, University of Toronto, Toronto, Ontario, Canada.

Fragile X syndrome (FRAXA) can be caused by either a deletion or a point mutation within the non-repeating region of the FMR1 gene, or more commonly by large expansions of the CGG repeat tract within the FMR1 promoter. The genetic instability of the FRAXA (CGG)_n repeat is sensitive to the length, purity and CpG methylation of the repeat tract. Tracts 25 pure CGG repeats can be unstably transmitted. Tracts that are 40-60 repeats long can be consistently inherited without mutations while in other families the same tract lengths can frequently lead to length changes. The different genetic instabilities of these Gray-Zone tract lengths is attributed to the presence or absence of AGG interruptions. Thus, expanded tracts that are interrupted with AGG are more stable than pure tracts of similar lengths. It has been suggested that loss of the AGG interruptions, by some yet unknown process, may be a precursor to expansion.

Through the use of bacterial and SV40 primate replication systems it was possible to study the effect of replication and CpG methylation upon repeat stability, as well as the sequence integrity, of a FRAXA clone with an interrupted gray-zone allele length of (CGG)₉AGG(CGG)₉AGG(CGG)₃₂ flanked by non-repeating genomic sequence. The presence of AGG interruptions increased the stability of the CGG repeat during replication, regardless of CpG methylation status. Deletions were predominantly limited to the repeat tract. However, rare deletion events involving the loss of repeats as well as the non-repeating flanking sequence 5' of the CGG tract were observed in CpG methylated templates replicated in primate cells. Interestingly, the 5 breakpoints of these deletions mapped to the deletion hotspots identified in some FRAXA patients. These results may explain the observation of FRAXA patients harboring deletions into the region flanking the CGG repeat, as well as upon how AGG interruptions might be lost.

Up-regulated genes after demethylation corresponds to regions of recurrent chromosomal breakpoints and fragile sites In Osteosarcoma. *K. Al-Romaih^{1,3}, I. Braude^{1,3}, J. Bayani³, P. Marrano³, G. Lim³, S. Selvarajah^{1,2}, B. Beheshti³, P. Thorner^{1,2}, M. Zielenska^{1,2}, J. Squire^{1,3}.* 1) Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada; 2) Department of Pediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, Canada; 3) University Health Network, Ontario Cancer Institute, Princess Margaret Hospital, Toronto, Canada.

A large number of translocations take place in Osteosarcoma (OS) and often the breakpoints occur in regions of the genome containing fragile sites (FS). It has also been suggested that FS regions may be subject to epigenetic modification during tumorigenesis. This prompted us to profile epigenetic change in OS and to determine whether differential methylation of specific loci also corresponded to the regions of high chromosomal rearrangements in this tumor. We employed expression gene-chip, and CpG island arrays to identify chromosome regions corresponding to differential gene expression in human OS cell lines U2OS and MG63 and normal human osteoblasts after treatment with the de-methylation agent 5-aza-2deoxycytidine. We also correlated the regions of changes in gene expression to chromosomal FS. Our data show that up-regulated genes, and areas of differential expression, after de-methylation, map to the same specific chromosome regions recurrently rearranged in human OS cell lines. We also found closer correlation between regions of up-regulated genes after 5Aza-dc treatment and FS (cytobands 1p36, 1q12.32, 2p13, 6p22-23, 9p21, 14q23-24). This study provides further support for the role of epigenetic modification at distinct chromosomal regions of the OS genome. Significantly, the concordance between areas of the genome that are subject to differential methylation, and the location of recurrent chromosomal rearrangements and FS suggests that differentially expressed genes, associated with oncogenesis may cluster to distinct chromosomal domains that have a propensity to undergo translocation during tumor progression.

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Gene inactivity leads to permanent silencing of the *RARB2* tumor suppressor gene. *G. Bistulfi, M. Ren, S. Pozzi, G. Somenzi, N. Sacchi.* Cancer Genetics, Roswell Park Cancer Institute, Buffalo, NY.

In cancer cells genes become silenced in association with repressive epigenetic changes such as histone modifications and DNA hypermethylation. This is the case for the tumour suppressor gene retinoic acid receptor beta 2 (*RARB2*) that mediates the action of retinoic acid (RA), the bioactive derivative of vitamin A. *RARB2* is the target gene of another RA-receptor, RAR alpha (*RARA*). In the absence of RA, a *RARA*-RXR heterodimer binds to a RA-responsive element (beta RARE) and along with co-activators maintains the beta- RARE-containing chromatin region in a poised, transcriptionally permissive state. Upon RA binding to *RARA*, additional co-activators, histone acetyltransferases, and ATP-driven chromatin remodeling activity are recruited at *RARB2* in a stepwise fashion leading to *RARB2* transcription. Cancer cells are often RA resistant because of *RARB2* silencing. *RARB2* silencing has been traced to repressive modifications including DNA hypermethylation and histone modifications. We hypothesised previously that an aberrant *RARB2* inactive status can attract repressive histones and DNA changes, leading to permanent silencing (Sirchia et al., 2000; 2002). We tested this hypothesis by impairing *RARA* by using both genetic and pharmacological strategies. Abrogation of *RARB2* transcriptional activity was paralleled by the induction of histone and DNA and modifications at *RARB2*. Loss of *RARB2* was concomitant with in vitro tumour phenotypic changes and RA resistance. Acknowledgements: This work was supported by US Army IDEA DAMD17-02-1-0432 Award, RPCI Alliance Foundation and AROCC Foundation.

Analysis of DNA Methylation in the FSHD Syndrome-linked *D4Z4* Repeat with a New Highly Specific Probe. *M. Ehrlich*¹, *K. Jackson*¹, *M.E. Ballestas*¹, *G. Jiang*¹, *S. Winokur*², *V. Vedanarayanan*³. 1) Depts. of Biochemistry and Human Genetics, Tulane Medical School, New Orleans, LA 70112; 2) Dept. of Biol.Chem., Univ. of Cal., Irvine CA 92697; 3) Dept. of Neurol., Univ. of Miss., Jackson MS 39601.

Facioscapulohumeral muscular dystrophy (FSHD) is characterized by the shortening of an array of 3.3-kb tandem repeats called *D4Z4*. Though this array is on the subtelomeric regions of both 4q and 10q, only shortening of the 4q array is associated with the disease. The presence of many *D4Z4*-like sequences elsewhere in the genome has interfered with methylation analysis of *D4Z4* by Southern blotting. Therefore, we tested subfragments of the 3.3-kb repeat to try to find a hybridization probe that is specific for the *D4Z4* sequences on chromosomes 4 and 10. By Southern blot analysis of DNA from monochromosomal human-rodent somatic cell hybrids (SCH), we demonstrated that a 1-kb subfragment of *D4Z4* has high specificity for the *D4Z4* repeats on chromosomes 4 and 10. In contrast, when we tested PCR primer-pairs from six different subregions of *D4Z4* for amplification of a panel of SCH DNAs, none was specific for chromosomes 4 and 10. Using the 1-kb *D4Z4* subfragment as a hybridization probe, we analyzed *D4Z4* methylation at CpG methylation-sensitive sites for *SmaI* and *EagI* in various control tissues, including skeletal muscle biopsy samples. All of these DNAs showed much methylation at *D4Z4*, which contains an unusually high CpG content (73% CpG). However, an appreciable amount of signal was also in lower-molecular-weight restriction fragments. There were similar patterns of methylation at *SmaI* sites in two control and two FSHD skeletal muscle biopsy samples and in control and FSHD myoblast cell strains, whose cell type was confirmed by desmin staining. Several control and FSHD lymphoblastoid cell lines showed moderate hypomethylation in *D4Z4* compared to the other samples. This new hybridization probe will be useful not only for *D4Z4* methylation analysis, but also for analysis of 4q or 10q-specific *D4Z4* transcripts and nuclease sensitivity of *D4Z4* chromatin. This research was supported in part by NIH Grant R21 NS43794.

RASSF2A, a member of the RASSF family of putative tumour suppressor genes, is inactivated in colorectal and lung cancer by promoter hypermethylation. *L. Hesson¹, M.D. Vos², D. Morton¹, E.R. Maher¹, G.J. Clark², F. Latif¹.*
1) Medical & Molecular Genetics, University of Birmingham, Birmingham, United Kingdom; 2) Department of Cell and Cancer Biology, National Cancer Institute, National Institute of Health, Rockville, Maryland, USA.

The RASSF1A tumour suppressor gene, located at 3p21.3, is frequently inactivated by promoter region hypermethylation in a variety of human cancers including lung, breast, kidney, neuroblastoma and glioma. RASSF2, located at 20p13, is an additional RASSF member identified using in silico BLAST searches. RASSF2 has three isoforms designated RASSF2A, RASSF2B and RASSF2C. All three proteins contain a C-terminal Ras-association domain. Recently, we have shown RASSF2A protein to be a novel pro-apoptotic K-Ras effector that induces cell cycle arrest. RASSF2A was expressed in all tissues examined including lung. Since expression of RASSF2A is frequently downregulated in tumour cell lines we examined the RASSF2A CpG island for promoter hypermethylation. RASSF2A CpG island was hypermethylated in 56% breast, 45% SCLC, 100% NSCLC, 78% colorectal, 20% neuroblastomas, 67% glioma and 88% kidney tumour cell lines. Loss of or downregulated RASSF2A expression was shown in methylated lung and colorectal tumour cell lines and expression was restored after treatment with a demethylating agent. In primary tumours 44% NSCLC and 70% colorectal tumours underwent RASSF2A CpG island hypermethylation. Allele loss also occurs at the 20p13 region in around 30% of methylated NSCLC tumours. Growth suppression analysis of colorectal cell lines by RASSF2A and functional analysis using yeast two-hybrid assay is currently underway. Our results demonstrate that RASSF2A is inactivated in human cancers by CpG island hypermethylation. Furthermore our data warrants investigation of other RASSF family members for possible epigenetic inactivation and functional analysis.

X chromosome inactivation patterns in elderly females aged over 70 years. *I.Y. Iourov¹, L. Villard², S.G. Vorsanova³, I.A. Demidova³, S.A. Shalnova⁴, M.A. Shkolnikova³, A.M. Olferiev⁴, Y.B. Yurov¹.* 1) Molecular Genetics, National Center Mental Health, Moscow, Russian Federation; 2) INSERM U491, Faculte de Medecine, Marseille, France; 3) Institute of Pediatrics and Children Surgery Russian Ministry of Health, Moscow, Russia; 4) Center of Preventive Medicine, Ministry of Health, Moscow, Russia.

X chromosome inactivation is a transcriptional silencing of one X chromosome in mammalian females. This phenomenon leads to X-linked gene dosage compensation. In females the mean contribution from each parental chromosome X is 50%. However due to randomness of this process some deviation from the mean contribution is noticed. The study of X chromosome inactivation patterns in normal females showed conflicting results. Particularly higher percentage of elderly females with skewed X-inactivation was observed. These results have led to propose the hypothesis about age dependence of X chromosome inactivation patterns. Therefore it is of great interest to investigate X chromosome inactivation patterns in elderly females. In this study we have analyzed X-inactivation patterns in 40 female aged from 74 to 85 years (mean age 78 years). The control group was 36 females (mean age 30 years). The most common AR-assay (the study of methylation patterns of HpaII site of human androgen receptor gene (HUMARA) by quantitative PCR) to determine X-inactivation patterns was used. The age dependence of X-inactivation was not observed. We have detected skewed X-inactivation in three women among 40 (7.5%) elderly females comparing to two women among 36 (5.5%) females from control group. The difference was not found to be statistically significant ($p > 0.4$). We made a suggestion that higher incidence of skewed X-inactivation in elderly females revealed by previous studies could occur due to some experimental ambiguities as a heterogeneity of groups studied (in some studies females having relatives with genetic abnormalities associated with skewed X-inactivation patterns were included) and the difference of X chromosome inactivation skewing determination. We conclude that present study does not show X chromosome inactivation to be age dependent.

The dactylaplasia phenotype is suppressed by the silencing of its mutational retrotransposon. *H. Kano*¹, *H. Kurahashi*², *T. Toda*¹. 1) Division of Functional Genomics, Department of Post-Genomics and Diseases, Osaka University Graduate School of Medicine, Osaka, Japan; 2) Division of Molecular Genetics, Institute for Comprehensive Medical Science, Fujita Health University, Aichi, Japan.

Dactylaplasia, characterized by missing central digital rays, is an inherited mouse limb malformation that is clearly dependent on a two-locus interaction between *Dac* and *mdac*. The dactylaplasia phenotype depends on the genotype at the mutated locus, *Dac*, and also requires homozygosity for a recessive allele at another unlinked locus, *mdac*. Two independent *Dac* mutations in the *dactylin* gene on mouse chromosome 19 have been identified from different strains. Both mutations found in the *Dac* locus were insertions and were named *Dac1* and *Dac2*. However, little is known about the interaction between *Dac* and *mdac*. We therefore focused on the property of the unknown recessive alleles, *mdac* (which permits expression of *Dac*) and *Mdac* (which inhibits expression). We first cloned these insertions of 7,486bp for *Dac1* and 7,473bp for *Dac2*. The insertions were long terminal repeat (LTR) retrotransposons and were 99.6% identical. Most transposons are thought to be maintained in a silent state. Bisulfite sequencing revealed that CpG dinucleotides in the LTRs of mice carrying the *Mdac* allele were hypermethylated, although those of mice carrying only the *mdac* allele were hypomethylated. We also carried out ChIP analysis on nucleosomes of the LTR region and found that hypermethylated LTRs consisted of inactive chromatin, whereas hypomethylated LTRs consisted of active chromatin. In addition, these epigenetic changes caused by *Mdac* were limited to the 5LTR region. Our results indicate that some types of LTR retrotransposons are silenced by *Mdac*, leading to chromatin inactivation and DNA methylation at their 5LTR regions. Dactylaplasia will contribute to our understanding of the hosts defense mechanism for suppressing and silencing such retrotransposons.

Epigenetic implications in schizophrenia: site-specific cytosine methylation in S-COMT promoter on 22q11. B.C.

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The COMT gene on chromosome 22q11 remains a strong candidate gene for schizophrenia susceptibility due to its chromosome location and protein function. Further, the identification of a Val/Met polymorphism in exon 4 has been assessed in individuals with schizophrenia with conflicting results. We hypothesized that the COMT gene association with schizophrenia may not involve genetic alterations, rather the allele specific activity alterations could be brought about by epigenetic changes (epi-alleles) and may involve methylation. We have tested this hypothesis by examination of methylation profile of the human S-COMT promoter using sodium bisulfite. This genomic region overlaps with the MB-COMT coding region, includes 56 cytosines. The analysis of 31 adult brain regions and 51 individual blood samples suggests that the methylation is restricted to the CpG dinucleotides only; four sites (6, 17, 51 and 53) are completely methylated while two site (23 and 27) are partially methylated and differ from sample to sample. For example site 27 is partially methylated in three blood sample and five brain regions while site 23 is partially methylated in all samples with one exception. The exception being a blood sample from a schizophrenia patient with predominant negative symptoms. Interestingly, three pairs of monozygotic twins discordant for schizophrenia showed no differences in methylation in this region. The results offer three conclusions. It may be possible to use blood DNA in methylation studies. The results rule out a role for DNA methylation in the S-COMT promoter in discordance of MZ twins for schizophrenia. Finally, complete methylation of cytosine 23 in one case of schizophrenia may suggest that it may represent one, but not a common cause of this heterogeneous and complex disease. Financial support for this research was provided by Ontario Mental Health Foundation and the Bill Jefferies Schizophrenia Endowment Fund.

Microarray- based large scale profiling of DNA methylation. *A. Schumacher*¹, *P. Kapranov*², *J. Flanagan*¹, *Z. Kaminsky*¹, *A. Assadzadeh*¹, *P. Yau*³, *C. Virtanen*³, *N. Winegarden*³, *T. Gingeras*², *A. Petronis*¹. 1) The Krembil Family Epigenetics Laboratory, Centre for Addiction and Mental Health, 250 College St, Toronto, ON, Canada M5T 1R8; 2) Affymetrix, Inc. 3380 Central Expressway Santa Clara CA 95051, USA; 3) The Microarray Centre, The Ontario Cancer Institute, 610 University Avenue, Toronto, ON, Canada M5G 2M9.

We describe a microarray technology that enables DNA methylation profiling of large chromosomal regions. The technology consists of two steps: i) enrichment of methylated- and unmethylated- DNA fractions, and ii) hybridization of the enriched DNA-fractions on high-density arrays. For the enrichment procedure, genomic DNA is treated with methylation sensitive and insensitive restriction enzymes, ligated to universal adaptors, and subjected to polymerase chain reaction that preferentially amplifies either unmethylated or methylated DNA fragments. Amplification products are then hybridized to microarrays to detect DNA methylation differences between tester and control samples. Various aspects of the technology including replicability, informativeness, sensitivity, and the minimal amount of template DNA, among others, were investigated using a microarray containing ~400 oligonucleotides that represent ~100 kb of genomic DNA and also a 12,192 element- containing CpG island microarray. Epigenetic profiling at the chromosome-wide scale is demonstrated using high-density chr 22 microarrays that consists of 337,256 probe pairs. The method represents a valuable tool in studying epigenetic variation in normal and pathological conditions over large genomic fragments. The principles developed in this work in addition to the availability of high-resolution microarrays for all human chromosomes will make profiling of DNA methylation of the entire human genome a routine procedure.

Program Nr: 1122 from the 2004 ASHG Annual Meeting

Analyses of epigenetic DNA modification in the human genome. *Y. Tamura, H. Kunugi, K. Kaneko, H. Hohjoh.* Nat. Inst. Neuroscience, NCNP, Kodaira, Tokyo, Japan.

Epigenetic genome modification such as DNA methylation appears to be involved in the regulation of gene expression, chromatin structure and genomic imprinting, and also associated with various diseases. The level of such a modification on the genome is difficult to determine and still poorly understood. In this study we established a method, by which the relative levels of DNA methylation at a methylation sensitive restriction enzyme site in genomic DNA can be determined. This method depends on methylation sensitive restriction enzymes and a real-time PCR. Using the method, we attempted to investigate the levels of DNA methylation at a methylation sensitive enzyme (BssH II) site in the human REELIN (RELN) and dopamine D2 receptor (DRD2) genes. Results indicate that the levels of DNA methylation at a BssH II site in the RELN gene vary among individuals, whereas the levels of DNA methylation at a BssH II site in the DRD2 gene appear to be similar among them. These observations suggest that genes undergoing either similar or different levels of DNA methylation among individuals are present in the human genome.

Molecular mechanisms for Myotonic Dystrophies (DM) 1 and 2: the role of RNA CUG and CCUG-binding proteins. *L.T. Timchenko*¹, *E. Salisbury*², *B.G.H. Schoer*³, *C. Schneider-Gold*⁴, *N.A. Timchenko*². 1) Dept Cardiovascular Sci, Baylor Col Medicine, Houston, TX; 2) Huffington Center on Aging, Baylor Col Medicine, Houston, TX; 3) Friedrich-Baur-Institute, LMU, Munich D-80336, Germany; 4) Neurochirurgische Universitaetsklinik, Wuerzburg 97080, Germany.

DM1 is a neuromuscular disease caused by an untranslated CTG repeat expansion on chromosome 19q. A new type of DM, DM2, is associated with an untranslated tetranucleotide CCTG repeat expansion on chromosome 3q. In DM1, CUG repeats affect two distinct families of CUG RNA-binding proteins, CUGBP1 and MNBL, contributing to the myotonia, insulin resistance, muscular dystrophy and delay of muscle differentiation and development. Since GC-repeats are present in CUG and CCUG repeats, the same RNA GC-binding proteins might be affected in both diseases. We found that CUGBP1 activity and protein levels are elevated in both DM1 and DM2. This observation suggests that, in DM2 patients, CCUG repeats might disrupt specific CCUG-binding proteins. To identify novel CCUG RNA-binding proteins, we used a combination of chromatography columns (HPLC). We have purified to homogeneity a Mega Protein-Protein Complex (MPPC) that specifically interacts with long CCUG repeats. Detailed analysis of this complex shows the MPPC has a molecular weight 1 million Dalton and consists of 10-12 proteins. UV cross-link determined three RNA-binding proteins within this complex. Immunoblotting with monoclonal antibodies revealed that one of the CCUG binding proteins is CUGBP1. Protein sequencing of the other components of the MPPC is on the way to determine a biological role of the complex in normal cells and examine if this function is affected in DM2. Investigations of RNA binding proteins in DM1 using a CUG riboprobe also identified high molecular weight CUGBP1-protein-protein complexes that bind preferentially to CUG repeats. These data are consistent with the hypothesis that expanded RNA CUG and CCUG repeats might destroy biological functions of multimeric protein-protein complexes leading to the complex phenotype of DM1 and DM2 diseases.

Withdrawal from the cell cycle in FSHD myoblasts. *D.A. Figlewicz, Y. Hong, I. Coltas.* Dept Neurology, Univ Michigan, Ann Arbor, MI.

Facioscapulohumeral dystrophy (FSHD) is an autosomal dominant disorder resulting from deletion of 3.3 kb ("D4Z4") repeats on chromosome 4q35. In recent years, our attention has focused on studies of FSHD myoblasts, which display an aberrant phenotype in both the undifferentiated and differentiated states. FSHD myoblasts upregulate the cyclin dependent kinase inhibitor, p21, divide less robustly, and leave the cell cycle and fuse more readily than controls. While p21 is upregulated downstream of MyoD and initiation of the myogenic differentiation program in control myoblasts, we have determined, using real-time PCR, that the p21:MyoD ratio is nearly 3 fold elevated in FSHD myoblasts. This suggests that a pathway other than normal differentiation may be responsible for withdrawal from the cell cycle in FSHD myoblasts. Recent identification of D4Z4-binding proteins has suggested a new direction for study. The D4Z4-binding proteins include YY1, a member of the Class I polycomb protein complex. Acetylation of members of the Class I polycomb complex leads to activation of the Class II group of polycomb group proteins, which localize to the surface of condensed chromatin domains. Perhaps the functionally most important member of this group is the polycomb protein Bmi1. Not only is Bmi1 association with chromatin and heterochromatin inversely related to progression of the cell cycle, it has been shown to promote proliferative capacity in several cell types, including human fibroblasts. Nothing is known about the potential role of Bmi1 in control of myoblast proliferation. We quantitated expression levels of Bmi1 in undifferentiated control and FSHD myoblasts; Bmi1 expression is significantly decreased in FSHD myoblasts. Bmi1 in other cell types suppresses the expression of tumor suppressor protein p16. We are currently investigating whether p16 mRNA and/or protein levels are altered in undifferentiated FSHD myoblasts.

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Etiology of epigenetic gene silencing in cancer cells. *M.Q. Ren, S. Rossetti, G. Bistulfi, S. Pozzi, G. Somenzi, N. Sacchi.* Cancer Genetics, Roswell Park Cancer Institute, Buffalo, NY.

In non-embryonic normal cells DNA methylation of cytosine of the dinucleotide sequence CpG is distributed globally in the genome with exception of CpG islands. In contrast, aberrant hypermethylation of CpG islands that have been associated with loss of transcription of genes essential for crucial biological functions has been detected in virtually every type of cancer. DNA hypermethylation in cancer cells is likely the tip of the iceberg of hierarchical epigenetic changes in somatic cells. We hypothesize that aberrant gene inactivity can attract hierarchical chromatin repressive changes (at both histone and DNA level) ultimately leading to profound gene downregulation and stable gene silencing. To test whether aberrant gene inactivity can attract repressive chromatin changes at both histone and DNA level we used different somatic cell systems and induced aberrant transcriptional repression of target genes of a) nuclear receptors and b) fusion proteins. Induction of aberrant transcriptional repression led to both repressive histone modifications and DNA hypermethylation with consequent gene downregulation and stable gene silencing. These epigenetic changes were concomitant with biological changes indicative of tumor progression.

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Epigenetic consequences of genetic abnormalities in leukemia. *S. Rossetti*¹, *A.T. Hoogeveen*², *N. Sacchi*¹. 1) Cancer Genetics, Roswell Park Cancer Institute, Buffalo, NY; 2) Erasmus Medical Center, Rotterdam, The Netherlands.

Leukemia chromosomal abnormalities often result in the production of aberrant fusion proteins. The translocation t(16;21)(q24;q22) often associated with therapy-related acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) leads to the ectopic production of the AML1-MTG16 fusion protein. This protein retains the DNA binding domain of AML1, a transcription factor crucial for normal hematopoiesis, and most of the functional domains of MTG16, encoding a transcriptional corepressor able to recruit histone deacetylase (HDAC) activity. For this reason, we hypothesized that AML1-MTG16 can aberrantly regulate AML1 target genes by inducing repressive epigenetic modifications of histone tails. To test this hypothesis, we used a cellular system (32D cells) where AML1-MTG16 induces block of granulocytic differentiation and inhibition of cell proliferation and we examined the chromatin associated with AML1-target genes. AML1-MTG16 expression in 32D cells results in down regulation of the endogenous *Mpo* and *Csf1r*, two AML1-target genes, which are relevant in the myelogenesis process. Interestingly, by using chromatin immunoprecipitation analysis the regulatory regions of the *Csf1r* locus were found significantly hypoacetylated at both histone H3 and H4. These results establish a mechanistic link between a myeloid-specific fusion protein and aberrant epigenetic regulation of a myeloid differentiation gene.

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Chromatin remodelling is involved in the pathogenesis of the Mandibuloacral Dysplasia (MADA). *F. Gullotta¹, G. Lattanzi², A.M. Nardone¹, M.R. D'Apice¹, I. Filesi³, C. Capanni², P. Sabatelli², G. Scarano⁴, S. Biocca³, N.M. Maraldi², G. Novelli¹.* 1) Biopathology, University of Tor Vergata, Rome, Italy; 2) Cell Biology and Electron Microscopy, Rizzoli Orthopedic Institute, Bologna, Italy; 3) Neuroscience, University of Tor Vergata, Rome, Italy; 4) Division of Medical Genetics, Gaetano Rummo Hospital, Benevento, Italy.

Chromatin remodelling influences DNA and histone modifications, which are responsible of chromatin structure and thereby gene expression, DNA methylation, replication, recombination, repair, apoptosis and senescence. Several evidences have established the relationship between epigenetic changes and the molecular pathogenesis of many monogenic diseases involving chromatin remodelling. The Mandibuloacral Dysplasia (MADA, OMIM 248370) is caused by mutation in LMNA-encoding lamin A/C. Lamins exert a profound influence in the maintaining the structural integrity of the nuclear lamina and on the organization of heterochromatin within the nucleus. We examined the nucleus morphology, the cytoarchitecture, the chromatin organization and the distribution of its associated proteins in fibroblast cell lines from 3 MADA patients carrying the R527H homozygous mutation. Nuclear alterations mostly consisting with focal absence of peripheral heterochromatin, invaginations of the nuclear envelope or thin papillary projections, were observed in MADA cells. A different distribution pattern of heterochromatin protein 1 (HP1) and histone H3 methylated at Lysine 9 (Me9H3) were found in nuclei from MADA patients compared to controls. The interplay between heterochromatin and lamin A is likely to involve the nuclear lamina protein all along its maturation process. To verify this hypothesis, we studied the redistribution of heterochromatin during lamin A precursor processing. We detected a reduced rate of lamin A precursor maturation with a significant accumulation of pre-lamin A in MADA cells. This suggests that pre-lamin A plays a major role in peripheral heterochromatin organization, likely interacting with silenced hyper-methylated chromatin and strongly supports a major role of the chromatin in the pathogenic pathway of MADA and possibly other laminopathies.

Tissue-specific shifting of early-late replication zone borders: a general mechanism for higher-order gene regulation. *R.S. Hansen, B. Huse, T.K. Canfield, K.R. Varadarajan, S.M. Gartler.* Medicine/Medical Genetics, Univ. Washington, Seattle, WA.

The genome is composed of a number of large replication time zones that correspond to the classical R and G chromosome bands. Using a bromodeoxyuridine-based molecular replication timing assay, we examined replication timing patterns in several cell types for STS markers in two late-replicating G band regions, Xq27 and 11p15.4, and in their flanking early-replicating R bands. Although cytogenetically determined replication banding patterns are thought to be constant over tissue type, we found that the molecularly defined borders of the corresponding replication time zones differed between cell types. These border shifts of one to several megabases correlate with cell type-specific expression of a subset of genes in the interborder region such that these genes have the classic pattern of early replication in expressing cells and later replication in nonexpressing cells. The beta globin gene cluster on 11p15.4, for example, is within a large chromosomal segment of late replication in nonexpressing cells of about 1 Mb in size. In expressing erythroleukemia cells, this band shows a dramatic shift in early-late replication border locations in that the entire zone shifts to early replication. Thus, either one or both of the replication borders is altered in expressing cells so as to advance replication of the entire band. Gene regulation by border shifting is also seen in fragile X syndrome in that we previously found *FMRI* repression to be associated with a variable border shift that always brings the gene into a large zone of very late replication that is normally proximal. The general phenomenon allows for an efficient way to package genes that are near replication time zone borders into a late-replicating, repressive domain, and suggests that tissue-specific genes depending on late replication for full repression might be preferentially located near replication borders. We speculate that replication border shifting, if general, could have implications for higher-order gene regulation, genome organization, and abnormal epigenetic states associated with disease.

The effect of the Z-DNA structure of genomic retroelements in major psychosis and stressed chickens on the experimental design of epigenetic studies. *P.X. Kan^{1,2}, X.G. Yang², D.J. Zhang², Z.L. Xie³, S.C. Wang², W.Y. Huang².*

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Elucidation of the causes of schizophrenia (SCH) and bipolar disorder (BD) remains to be the main challenge to psychiatric researchers. Despite strong evidence of the importance of hereditary factors in SCH and BD as well as significant effort to identify disease genes, the progress in the field of molecular psychiatric genetics has been quite slow. This work is dedicated to the exploration of the role of epigenetic (epiG) factors in major psychosis. One of the key functions of epigenetic modification of the genome of eukaryotic cells is to suppress transcriptional activity of the retroelements. Examples of retroelements are human endogenous retroviruses (HERV) sequences, Alu elements and long interspersed nucleotide elements (LINE), among others, which as a rule are hypermethylated. There is evidence from schizophrenia (SCH) and other human complex diseases that some of the genomic retroelements become transcribed in the affected tissues. Our goal was to screen DNA samples from post-mortem brain tissues of individuals who were affected with major psychiatric illness for retroelements that were located in the hypomethylated fraction of the genomic DNA. The HERV sequences in the hypomethylated fraction of brain DNA were below the detection level. Over 100 Alu sequences were cloned, sequenced, and mapped to the human genome. The results were, however, not consistent. The possibility is that the HERV sequences might locate in the DNA breakpoint region with Z-DNA structure in high salt solution. The human postmortem brain genomic DNA was severely degraded. The comparison of postmortem brain genomic DNA between human and chickens showed that the degree of degradation of brain genomic DNA increase with postmortem interval. Better experimental design is required to produce consistent result in epigenetic studies.

Transcriptional Regulation of ETn Elements: Active Mobile Mutagens in the Mouse. *I.A. Maksakova*^{1,3}, *D.L. Mager*^{1,2}. 1) Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada; 2) Department of Medical Genetics, UBC; 3) Genetics Graduate Program, UBC.

Novel insertions of endogenous retroviruses (ERVs) account for up to 10% of spontaneous mutations and numerous cases of cancer in mice. Our research focuses on the early transposons (ETns), a class of mouse ERVs highly transcribed during early mouse embryogenesis and in undifferentiated cells. Flanked by long terminal repeats (LTRs), ETns do not contain retroviral open reading frames (ORFs), but remain transcriptionally active, inducing insertional mutations. ETns have a close relative in the mouse genome, a full-length ORF-possessing mouse ERV, MusD. There is at least twice the copy number of MusD compared to ETnII elements; however, ETnII elements account for almost 30 times more transcripts, despite the fact that ETnII and MusD LTRs, carrying transcription control elements, are closely related. The objective of this research is to determine the factors responsible for the difference in transcription level between ETnII and MusD elements and to elucidate the reason for preferential embryonic expression of ETns. Reporter gene assays demonstrate that ETnII LTRs exhibit greater promoter ability in comparison to MusD LTRs, and their activity in undifferentiated embryonic carcinoma P19 cells is considerably higher than in differentiated NIH/3T3 cells. Electrophoretic mobility shift assay (EMSA) utilizing DNA probes specific to the only region of significant divergence between ETnII and MusD LTRs confirms that different transcription factors (TFs) bind this region. Deletion experiments helped identify an 80 bp region responsible for high LTR promoter efficiency in P19 cells, confirmed by EMSA to bind Sp1 and Sp3 TFs. Southern blotting suggests lower methylation levels of ETnII LTRs in P19 cells compared to NIH/3T3 cells. In conclusion, differences in the LTRs of ETnII and MusD elements seem to account for differences in their expression level. Both types of LTRs harbour cis-acting elements responsible for their preferential transcription in undifferentiated cells. Finally, the embryonic pattern of ETn expression appears to be influenced by both methylation and TF regulation.

Possible epigenetic protective factor for FSHD identified in the Black South African population. A. Olckers^{1,2}, A. Van der Merwe^{1,2}. 1) Centre for Genome Research, North-West University, Pretoria, South Africa; 2) DNAbiotec (Pty) Ltd, Pretoria, South Africa.

Facioscapulohumeral muscular dystrophy (FSHD) is the third most common inherited disorder of muscle after Duchenne and Myotonic dystrophy, with a prevalence of at least 1 in 20,000. FSHD is characterised by progressive weakening and atrophy of the face, shoulder-girdle and upper arm, although other skeletal muscles may also become involved with progression of the disorder. This disorder has a highly variable phenotypical presentation with weakness occurring from infancy to late life, but generally in the second decade of life. The FSHD phenotype segregates as an autosomal dominant trait. A deletion of an integral number of 3.3 kb repeats on chromosome 4q35, localised at the D4Z4 locus, was reported to cause FSHD. Translocation events between chromosomes 4q35 and 10q26, also harbouring similar 3.3 kb repeat units, have been detected via the presence of Bln I sites within the 10q26 repeats. In this study the D4Z4 locus was investigated for the first time on a molecular level in the Black South African population. The translocation frequency between chromosomes 4q35 and 10q26 was evaluated via the Bln I / Bgl II dosage test. The Eurasian population harbours an excess of 4-on-10 fragments and this excess was postulated to be a significant, if not the major predisposing factor that gives rise to the FSHD-type deletion. The predisposed population thus has individuals that are more susceptible to FSHD. An enrichment of 10-on-4 was observed in the Black South African population. It was postulated that this enrichment is an epigenetic protective factor for FSHD, since no FSHD case has been reported in this population to date. It was further hypothesised that the absence of FSHD cases in this population is due to this enrichment. As a consequence, the excess / enrichment of specific translocation profiles in different populations is an additional factor that affects the aetiology of FSHD within specific populations. We acknowledge the donation of the p13E-11 probe by S.M. van der Maarel and R.R. Frants, Dept. of Human Genetics, Leiden University Medical Centre, The Netherlands.

A powerful biological, clinical and diagnostic method to evaluate X-chromosome inactivation by RNA FISH. *T. Kubota*¹, *T. Nakamura*², *M. Mimaki*³, *H. Ohashi*⁴, *M. Tsukahara*⁵, *S. Yamamori*². 1) Epigenetic Medicine, Fac Med, Univ Yamanashi, Yamanashi, Japan; 2) Mitsubishi Kagaku Bio-Clinic Lab, Inc., Tokyo, Japan; 3) Saitama Child Med Ctr, Saitama, Japan; 4) Natl Inst Neuroscience, NCNP, Tokyo, Japan; 5) Fac Health Sciences, Yamaguchi University Sch Med, Ube, Japan.

X-chromosome inactivation (XCI) is a genetic phenomenon in females in which one of two X chromosomes is randomly inactivated. DNA methylation-based assays have recently been used to evaluate XCI in various genetic diseases. However, these assays detect abnormal XCI indirectly utilizing DNA methylation, and they are not capable of assessing XCI at each cell level. Here we show a new expression-based direct and cell-level XCI method using RNA FISH with XIST (X-chromosome inactive specific transcript) DNA probe. In this method, one XIST RNA signal derived from the inactive X chromosome was obtained in the nucleus of each cell of a normal female, and the RNA signal was merged with one of two XIST DNA signals from the two X chromosomes. By this method, we demonstrated that 1) two of three X had an XIST RNA signal in a XXX patient and three of four X had an XIST RNA signal in a XXXXY patient, indicating that Lyon's hypothesis can be visualized, 2) a 46,X,i(Xq)(10) patient showed no XIST RNA signal on the normal X whereas two XIST RNA signals on the i(X), suggesting that XIST gene is not inactivated by intrinsic XIST transcript from another XIST gene in cis, 3) a ring X Turner syndrome [46,X,r(X)] patient with severe mental retardation (MR) had no XIST RNA signal in all cells (i.e. complete functional X disomy), whereas a patient with the same karyotype but with less severe MR had some XIST RNA-positive cells signal (i.e. partial functional X disomy). These results indicate that the RNA FISH-based XCI assay will be an alternative powerful biological, clinical and diagnostic tool.

Analysis of concordance of X-inactivation patterns in haematopoietic and non-haematopoietic tissues of neonates and elderly females. *S. Provost¹, V. Bolduc¹, MP. Dube², C. Belisle¹, M. Gingras¹, P. Chagnon¹, L. Busque¹.* 1) Research Center, Maisonneuve-Rosemont Hospital, University of Montreal, Montreal, Canada; 2) Montreal Heart Institute, University of Montreal, Montreal, Canada.

Studies have shown that there is great variability of X-inactivation patterns in different tissues of a same individual. The incidence of inactivation skewing is generally greater in haematopoietic cells than in other tissues (epithelial cells, muscle, glandular tissue). One likely explanation is that as women age, the incidence of X-inactivation skewing in blood cells increases (acquired skewing phenomenon, Busque *et al*, Blood, 1996) and deviates from the initial X-inactivation pattern common to all tissues. To test this hypothesis, we compared the concordance of X-inactivation patterns between epithelial and blood tissues in cohorts of neonates and elderly females. Blood samples and buccal epithelium cells from 350 neonates and 648 females aged 55 years and over were analyzed for X-inactivation status using the HUMARA assay. In neonates, the incidence of skewing (using a criteria >3:1) was similar for blood and epithelial cells (10.6% and 7.7% respectively). Furthermore, the intra-individual correlation of X-inactivation ratios considering the direction of skewing was very strong ($r=0.71$, Pearson, $p<0.01$). In contrast, the incidence of skewing in the elderly cohort was significantly higher for blood cells than for epithelial cells (47.3% and 13.3%). The correlation between these two tissues in the elderly cohort was much lower ($r=0.59$, Pearson, $p<0.01$). These results suggest that the incidence of non-random X-inactivation at birth is low and concordant in different tissues. The concordance of X-inactivation patterns is lost over time as blood cells become progressively skewed, explaining the generally reported intra-individual discordances. The low and similar incidences of skewing in epithelial cells of neonates and elderly females suggest that skewing in this tissue is stable over time and correspond to the initial body-wide skewing. This also suggests that the increased incidence of skewing documented in blood cells of elderly females is acquired and specific to haematopoietic cells.

Analysis of 2,903 single base substitutions from the coding regions of 118 alternatively spliced genes associated with human disease. *F. Ma*¹, *L. Chen*², *H. Huang*², *J. Li-Ling*³, *X. Ji*^{4,5}, *Q. Li*¹, *Y. Wang*², *Y. Li*⁵. 1) School of Life Science, Liaoning Normal University, Dalian 116029, CHINA; 2) School of Life Science, Xiamen University, Xiamen 361005, CHINA; 3) Department of Medical Genetics, China Medical University, Shenyang 110001, CHINA; 4) Laboratory for Conservation and Utilization of Bio-resources, Yunnan University, Kunming 650091, CHINA; 5) Institute of Bioinformatics, Tsinghua University, Beijing 100084, CHINA.

Extensive interest has been expressed over the molecular mechanism and evolution of alternative splicing leading to human diseases. We have analyzed 2,903 single base substitutions from the coding regions of 118 alternatively spliced genes associated with human diseases retrieved from the Swiss-Prot, Human Gene Mutation Database and Human Gene Nomenclature database. Our results show that the proportions of substitutions are A/G or G/A, 28.14%; C/T or T/C, 31.97%; G/T or T/G, 13.65%; C/G or G/C, 12.47%; A/C or C/A, 7.44 %; and A/T or T/A, 6.34%, and those of the first, second and third bases of codons are 49.26%, 42.16% and 8.58%, respectively. There is an overall preponderance of transitions, i.e., A/G and C/T substitutions, over transversions (60.11% vs. 39.89%). The hierarchy of nucleotides undergoing substitution at the first, second and third bases of codons are C>G>A>T, G>C>T>A, and C>G>T>A, respectively. A rather high GC content in the coding regions was noticed. With mean substitution ratios being at 10.47%, 84.38%, 5.15%, respectively, for the high (65~81.06%), medium (40~65%), and low GC ranges (16.67~40%), the influence of GC content on substitution rates seems to be quite obvious. Substitution rates seem to be influenced mostly by the nature of substituted bases, existence of CpG di-nucleotides, and nearest flanking nucleotides. Substitution rate in middle exons (1 per 101bp) is significantly lower than those in 5'- and 3'- adjacent exons (81bp and 82 bp, respectively). Furthermore, there is a strong negative correlation between exon length and the rate of substitutions ($r=-0.1192$, $P<0.0001$).

Program Nr: 1135 from the 2004 ASHG Annual Meeting

AMPGEN - Attribution of missing persons by genotyping: User-friendly software for DNA forensics and pedigree-based genetic databases. *W. Niu, P. Gopalakrishnan, G. Zhang, R. Chakraborty.* Center for Genome Information, University of Cincinnati, Cincinnati, OH.

DNA-based human identification problems relate to forensics as well as validation of pedigree-based genetic databases. These problems are generally of three categories: Transfer Evidence (i.e., DNA profile of a question sample is from a single source, and it matches the profile of a known subject); DNA Mixture (i.e., the evidence DNA profile is a mixture, and statistical supports for different scenarios of mixture origin are needed); and Kinship Analysis (i.e., the evidence profile is to be assigned to a missing family member, based on DNA profile of one or more relatives of the family). Statistical genetic algorithms for addressing these problems are well established, but the computational methods may be complex and varied, depending on the assumptions made, and databases used. AMPGEN is a user-friendly platform to carry out such computations, based on user-specified sets of assumptions, population databases, and degree of conservativeness. The modules of this software require minimal data entry (to avoid transcriptional errors), and minimal genetic expertise to formulate the problem, but they provide comprehensive analyses based on alternative methods that are rationalized by population genetic theory. Linked with validated worldwide databases, the robustness of computations can be checked against unknown population origin of the question sample. AMPGEN also produces a report of the computations, explained in easy to understand language, and is accompanied with hypertext help files providing theoretical backgrounds of the computations and concepts, along with their literature citation. The modules can be integrated into any laboratory information management system (LIMS) to check accuracy of pedigree relationships, presence of contamination due to mixture, and presence of duplicates or related individuals in large genotype databases. Currently structured for use with the CODIS STR loci, AMPGEN can easily accommodate any panel of genomic polymorphic markers. (Research supported by a grant from the Department of Development, State of Ohio).

Program Nr: 1136 from the 2004 ASHG Annual Meeting

Natural selection in the human genome: Introducing the Finch program and discoveries from whole genome datasets. *P. Sabeti*¹, *P. Varilly*¹, *B. Fry*², *E. Walsh*¹, *H. Hutcheson*³, *J. Roy*¹, *N. Patterson*¹, *T. Bersaglieri*⁴, *J. Hirschhorn*^{1,4,5}, *S. O'Brien*³, *D. Altshuler*^{1,5,6}, *E. Lander*^{1,7}. 1) Program in Medical and Population Genetics, Broad Institute, MIT, Cambridge, MA; 2) Physical Language Workshop, Media Lab, MIT, Cambridge, MA; 3) Laboratory of Genomic Diversity, National Cancer Institute, Frederick, MD; 4) Divisions of Genetics and Endocrinology, Childrens Hospital, Boston, MA; 5) Department of Genetics, Harvard Medical School, Boston, MA; 6) Departments of Medicine and Molecular Biology, Massachusetts General Hospital, Boston, MA; 7) Department of Biology, MIT, Cambridge, MA.

Recent breakthroughs in the identification and evaluation of single nucleotide polymorphisms in the human genome have given researchers the opportunity to carry out large-scale scans for signals of natural selection in the genome. With the data now available, we introduce the robust software package Finch that incorporates multiple tests for selection including our own Long-Range Haplotype (LRH) Test. Finch can handle whole genome datasets such as an entire release of the International Human Haplotype Map, allowing scientists to scan the genome in a single sitting or analyze and create images of their region of interest. We first show the efficacy of the program using previously known genes to be under selection including HBB, G6PD, CCR5, and LCT. We then show its use in analyzing a large-scale multi-population dataset, 160 genes in 3 populations. Finally we present examples drawn from the analysis of the public HapMap data, potentially identifying new regions under selection.

Program Nr: 1137 from the 2004 ASHG Annual Meeting

Evolutionary rates of essential and non-essential genes for fertility. *D.G. Torgerson, B.R. Whitty, R.S. Singh.*
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Sex and reproduction related genes have been shown to evolve rapidly across a variety of taxa, particularly for genes involved in male reproduction. However the exact mechanisms for rapid evolution may be complex and heterogeneous, as an essential reproductive function may be as evolutionarily important as viability. We calculated nucleotide sequence divergence in over 900 orthologous human and mouse sequences that were implicated to be important in either fertility or viability using mouse knockout mutation phenotypes. We compared the evolutionary rates of 1) genes affecting male or female fertility, 2) genes affecting one or both genders, 3) genes that are more or less essential for fertility, and 4) genes affecting fertility or viability. Our data suggest that in some cases genes essential for fertility are as evolutionarily conserved as those essential for viability, and we hypothesize that the rapid evolution of sex and reproduction related genes is facilitated through an increased specialization of reproductive function.

Effects of Natural Selection on Inter-population Divergence at Polymorphic Sites in Human Protein-Coding Loci.

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2784 single nucleotide polymorphisms (SNPs) in 396 protein-coding genes were analyzed with respect to gene diversity within samples of individuals representing the three major historic human populations (African, European, and Asian) and with respect to inter-population genetic distance. Reduced levels of both intra-population gene diversity and inter-population genetic distance were seen in the case of both SNPs in the 5' UTR and nonsynonymous SNPs causing radical changes to protein structure. Reduction of gene diversity at SNP loci in these categories was evidence of purifying selection acting at these sites, which in turn causes a reduction in inter-population divergence. By contrast, a small number of SNP loci in these categories revealed unusually high genetic distances between the two most diverged populations (African and Asian), suggesting that the latter loci may have historically been subject to divergent selection pressures. Interestingly, these cases include immune system genes, hormones and hormone receptors, and metabolic enzymes, which might plausibly have been subject to different selective pressures in different environments over the course of human evolution.

The C677T polymorphism in the Methylenetetrahydrofolate Reductase Gene in Rheumatoid Arthritis Mexican Patients. *J.P. Mena¹, I.P. Davalos^{1,2}, J.I. Gamez³, L. Gonzalez³, F. Munoz-Valle¹, M. Vazquez del Mercado¹, G. Martinez¹, L. Sandoval², L.E. Figuera^{1,2}, M. Salazar-Paramo^{1,3}.* 1) Centro Universitario de Ciencias de la Salud, Departamento de Fisiologia, Universidad de Guadalajara; 2) Division de Genetica, CIBO, IMSS; 3) Unidad de Investigacion Medica en Epidemiologia Clinica, HE, CMNO. Guadalajara, Jalisco, Mexico.

The C677T polymorphism in the 5,10-Methylenetetrahydrofolate Reductase (MTHFR) gene, is responsible of the thermolabile form of the enzyme, reduced availability of 5-methyltetrahydrofolate (circulating form of folate) and mild hyperhomocysteinaemia. Moderate hyperhomocysteinaemia related to folate deficiency has been described in patients with cardiovascular risk and also in patients with autoimmune diseases including Rheumatoid Arthritis (RA), suggesting that immune activation could be involved in the development of hyperhomocysteinaemia (Schroecksnadel 2003).

Objective: To determine the frequency of MTHFRC677T in patients with RA. Methods: Group RA consisted in 60 RA mestizo Mexican patients diagnosed according to the American College of Rheumatology 1987 (ARA) and Group M consisted in 140 normal mestizo Mexican individuals. Both groups were typed for the MTHFRC677T by PCR/RFLP Hinf I method. Results. Genotype frequencies GF % (n) in Group RA group were: CC 27% (16) CT 60% (36), TT 13% (8). and GF in Group M were CC 31% (44) CT 52% (72), 17% (24). Allele Frequencies AF % (n) were in Group RA C 57% (68) T 43% (52) AF in Group M C 57% (160), T 43% (120). Results: Interpopulation comparisons were similar (Chi square, 1.27, p 0.36) Conclusions: The were similar MTHFRC677T AF between the two groups.

Evolutionarily dynamic G-protein-coupled receptor regulates the development of human cerebral cortex. X.

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The mammalian cerebral cortex is characterized by complex patterns of anatomical and functional areas that differ markedly between species, but the molecular basis for this functional subdivision is largely unknown. We studied an inherited condition in which specific regions of the cortex are preferentially disrupted. Bilateral frontoparietal polymicrogyria (BFPP), a recessively inherited genetic disorder of human brain, shows severely abnormal architecture in the frontal lobes, suggesting that the BFPP gene is essential for normal development of the human frontal lobes. We have previously identified GPR56 as a causative gene for BFPP. In mouse, Gpr56 expression is found in the embryonic and adult brain specifically in areas consistent with early neurogenesis. The pattern of Gpr56 expression and the anatomy of BFPP imply that GPR56 most likely regulates the normal development of the human frontal lobes. The frontal lobes of the human brain are the most highly developed part relative to other animals and it has long been thought that the evolution of the frontal lobes parallels the development of human communication and civilization. Therefore GPR56, a gene regulates the frontal cortical development, is a candidate gene for involvement in the development of the human brain. Sequence analysis of GPR56 reveals substantial evolutionary differences between mammals and nonmammals in that the N-terminal domain that defines GPR56 is unique to animals with a cerebral cortex. In addition, evolutionary analysis of GPR56 revealed a pattern of molecular evolutionary constraint in the clade containing humans and chimpanzees that is different from that in other primates ($P < 0.05$) and may be due to directional selection. Thus, GPR56 is not only essential during human cerebral cortical development and patterning, but may have been important in the evolution of the cerebral cortex beginning in the human-chimpanzee common ancestor.

Maternal lineages and Alzheimer disease risk within the Old Order Amish. *J. van der Walt¹, S. Slifer¹, P.C. Gaskell¹, W.K. Scott¹, E.R. Martin¹, A. Crunk², D. Fuzzell², M. Creason¹, L. McFarland², K. Welsh-Bohmer³, S.R. Johnson³, C.E. Jackson⁴, C.C. Kroner¹, J.L. Haines², M.A. Pericak-Vance¹.* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN; 3) Department of Psychiatry and Behavioural Sciences, Duke University Medical Center, Durham, NC; 4) Scott & White, Temple TX.

The Old Order Amish, founded by a small number of Swiss immigrants, exist in culturally isolated communities across rural North America. The consequences of genetic isolation and inbreeding within this group are evident in increased frequencies of many monogenic diseases and several complex disorders. Conversely, the prevalence of Alzheimer disease (AD), the most common form of dementia, is lower within the Amish than in the general American population. Since mitochondrial dysfunction has been proposed as an underlying cause of AD and a specific haplogroup was found to affect AD susceptibility in Caucasians, we investigated whether inherited mitochondrial haplogroups affect risk of developing AD dementia in central Ohio and northern Indiana/southern Michigan Amish kin-groups. 95 independent matrilineages were observed across 9 large pedigrees and classified into 7 major European haplogroups. Haplogroup T is the most frequent maternal line represented overall in the Amish (35.4%) while observed in only 10.6% of outbred American and European populations. Furthermore, the frequencies of haplogroups J and K are lower (1.0%) than in the outbred data set (9.4-11.2%). Affected case matrilineages and unaffected control lines were chosen from pedigrees to test if specific haplogroups or SNPs confer risk of AD. We did not observe frequency differences between AD cases compared to controls overall or when stratified by sex. Therefore, we suggest that the genetic effect responsible for AD dementia in the affected Amish pedigrees is unlikely to be of mitochondrial origin and may be caused by nuclear genetic factors.

Reconstructing the genealogy of a BRCA1 founder mutation. *F. Marroni*¹, *G. Cipollini*², *F. Bonatti*², *P. Radice*³, *E. D'Andrea*⁴, *A. Contegiacomo*⁵, *J.E. Bailey-Wilson*⁶, *G. Bevilacqua*², *M.A. Caligo*², *S. Presciuttini*¹. 1) Center of Statistical Genetics, Univ. of Pisa, Italy; 2) Dept. of Oncology, Univ. of Pisa, Italy; 3) National Tumor Institute, Milan, Italy; 4) Dept. of Oncology and Surgery, Univ. of Padua, Italy; 5) U.O. Medical Oncology, II Univ. of Naples, Italy; 6) IDR, NHGRI, Baltimore, MD, USA.

The BRCA1*1499insA mutation was initially detected in three unrelated families from Tuscany, a region from central Italy, and was then reported in other Italian regions. Haplotype analysis was previously carried out with 4 closely linked markers in nine families, and the presence of a common compatible haplotype was determined. We have now extended the collection of independent pedigrees with this mutation to 14 families (7 from Tuscany, 6 from Northern Italy, and 1 from Southern Italy). We estimated the time to the most recent common ancestor (MRCA) of all mutation carriers using the length of the haplotype shared by pairs of individuals. DNA samples were obtained from 50 subjects (28 mutation carriers). Thirty-seven STR markers (DeCode map) were typed, spanning about 24 cM around the BRCA1 gene. Haplotypes were determined using Merlin 0.9 and GeneHunter 2.1. The length in cM of the haplotype shared by all possible pairs of mutation carriers provided a triangular matrix of haplotype sharing. We obtained the time to the MRCA of any two individuals, given the length of the shared haplotype and the recombination fractions between adjacent markers, by computer simulations. This allowed us to convert the matrix of haplotype sharing into a distance matrix, in which each element corresponded to the most likely number of meioses separating any two individuals (twice the number of generations since their MRCA). The genealogical tree of the affected haplotypes was then obtained by submitting this distance matrix to the program KITSCH of the package PHYLIP. The final estimated time to the MRCA of all families was 30.4 generations. Its standard error was obtained by bootstrapping. Assuming a generation time of 25 years, the common ancestor lived 75994 years ago.

Association among homocysteine gene polymorphisms (C677T, A1298C in MTHFR gene and 844ins86 in CBS gene) with atherosclerotic cardiovascular disease in Mexican patients. Z.G. Ruiz-Portillo¹, L. Gonzalez-Herrera¹, V. Suarez-Solis², H. Laviada-Molina², A. Gonzalez-delAngel³. 1) Genetics, C.I.R. Dr. Hideyo Noguchi, Merida, Yucatan, Mexico; 2) Faculty of Medicine, Autonomous University of Yucatan; 3) Department of research in Human Genetics. National Institute of Pediatrics.

Atherosclerotic coronary artery disease is the most common cause of Cardiovascular Diseases (CVD), which is the first cause of mortality in Mexico. Elevated homocysteine levels have been associated with increased risk for CVD. Molecular defects in genes encoding enzymes involved in homocysteine metabolism may account for hyperhomocysteinemia and CVD in some populations. Homozygous individuals for the allele C677T-MTHFR have a significantly higher risk for CVD, particularly in the setting of low folate status. The frequency of the allele C677T is one of the highest in our population. Therefore, in this study, we analyzed the role of these three polymorphisms and their interactions in association with CVD in Mexican patients. 52 subjects with atherosclerotic CVD, demonstrated by coronariography were the case group and 31 health subjects without coronary lesion also confirmed by cardiac catheterism, were included as the control group. Polymorphisms were determined by PCR-RFLPs. Allelic, genotypic and Haplotypic frequencies were compared between cases and controls using the statistical software EpiInfo 2000. Results showed a statistically significant difference for the C677T allele ($p=0.0014$, OR= 2.23, IC= 1.11-4.88) for the Homozygous TT genotype. ($p=0.005$) and the TT/AA haplotype ($p=0.0018$), therefore suggesting a positive association between this polymorphism and CVD. For the polymorphisms A1298C-MTHFR and 844Ins68-CBS, a statistically significant difference was not found between cases and controls ($p>0.05$). Our data suggests that the C677T polymorphism in MTHFR gene and the homozygosity for this allele are associated to CVD in our population, confirming that C677T allele and the TT genotype are risk factors for CVD.

Extensive and breed specific linkage disequilibrium in *Canis familiaris*. *E.A. Ostrander¹, N.B. Sutter¹, M.A. Eberle¹, H.G. Parker¹, B. Pullar¹, E.F. Kirkness², L. Kruglyak¹.* 1) Human Biology Division, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) The Institute for Genomic Research, Rockville, MD.

The 156 breeds of registered dogs in the U.S. offer a unique opportunity to map genes important in disease susceptibility, morphology, and behavior. Linkage disequilibrium (LD) is of current interest for its application in whole genome association mapping, since the extent of LD determines the feasibility of such mapping. We have measured LD at five loci, none of which is under obvious selective pressure, in the Akita, Bernese Mountain Dog, Golden Retriever, Labrador Retriever, and Pekingese breeds in order to evaluate the utility of association mapping in dogs. Approximately 20 unrelated dogs were genotyped in each breed at each of five loci on canine chromosomes 1, 2, 3, 34, and 37. At each locus SNPs and indels were discovered and typed by resequencing STSs spaced one, two, three, and six Mb apart. Distances were based on alignment between the human genome and TIGR's 1.5x shotgun Poodle sequence. Strikingly, LD in canines is much more extensive than in humans. The distances at which median D' falls to 0.5 vary from 500-800 Kb in the Golden Retriever and Labrador Retriever to 2 Mb in the Bernese Mountain Dog and Pekingese, and 3.7 Mb in the Akita. This compares with just 30 Kb in a human CEPH population. LD in dog breeds is 20-100 times more extensive than in human populations. These findings correlate with dog breed history. The Golden Retriever and Labrador Retriever are the most popular U.S. breeds, and their populations have never experienced bottlenecks, unlike the other breeds, all of which underwent tight population bottlenecks this century. With extensive LD in dogs, a correspondingly smaller number of markers will be required for association mapping studies as compared with humans.

Empirical evaluation of inferred population structure from Y-chromosomal haplotypes. *J.E. Ekins¹, J.B. Ekins¹, L. Layton¹, N. Myres¹, L.A.D. Hutchison¹, K. Hadley¹, U.A. Perego¹, J.L. Peterson¹, M.L. Lunt¹, S.S. Masek¹, A.A. Nelson¹, M.E. Nelson¹, K.L. Pennington¹, A. Sims¹, T. Tolley¹, A. Welch¹, S.R. Woodward¹.* ¹ Sorenson Molecular Genealogy Foundation, Salt Lake City, UT; ² Department of Micro and Molecular Biology, Brigham Young University, Provo, UT.

Human population dynamics is an area of active research, primarily addressing movement through the Pleistocene era. Investigations addressing modern histories have been more limited, but are valuable in multiple areas: disease linkage and association studies, constructing reference populations for forensic questions, and reconstructing personal histories. Herein we characterize a diverse set of 1895 samples from various world populations genotyped at 24 NRY STR loci, with documented paternal genealogies extending to an average of 7 generations (max. 15). Using the *structure* algorithm we describe a method to hierarchically decompose a population into increasingly homogenous groupings. Cluster membership is affirmed by pairwise analysis of inter-cluster vs. intra-cluster MRCA times, as well as molecular diversity measures. This population structure, inferred from the genotypes of living individuals, is compared to historical genealogical records where loose geographic correlations and paternal lineage designations are observed. The final clusters are indicative of relatively recent paternal lineages, with a median depth of ~40 generations within a cluster. The presented high-resolution statistical analysis makes it practical to use individual NRY haplotype data to distinguish between closely related individuals, in order to investigate relatively recent population histories.

Interpreting missense variants in Oculocutaneous Albinism Genes Tyrosinase and P Gene: an evolutionary approach. *M.S. Greenblatt¹, S. Duraisamy¹, C. McBride¹, J.P. Bond¹, W.S. Oetting².* 1) Univ of Vermont; 2) Univ of Minnesota.

Oculocutaneous Albinism (OCA) is characterized by lack of skin and eye pigment, UV sensitivity, predisposition to skin cancer, and developmental eye defects. OCA is caused by mutations in several genes, most commonly the tyrosinase (OCA1) and P genes (OCA2). Tyrosinase is the key enzyme catalyzing melanin pigment synthesis from tyrosine; the P gene encodes a transport protein. Understanding their intragenic conservation patterns can help to predict functionally critical amino acids (AA), where variants are likely to cause OCA. To assess quantitatively the predictive value of AA conservation we have: 1) made sequence alignments and phylogenetic trees of the tyrosinase and P genes, 2) computationally studied their intragenic AA conservation patterns, and 3) tested how well the patterns predict OCA-associated and non-disease associated variants, using the Albinism database (<http://albinismdb.med.umn.edu>).

RESULTS: Evolutionary variation in the existing database of sequences is sufficient to determine statistically significant conservation of codons for tyrosinase but not for P gene. The SIFT program correctly predicted 82 % of the OCA1 associated tyrosinase variants and both of the non-OCA-associated polymorphisms, and 94% of the OCA2-associated P gene variants. However, 4 of 6 P gene polymorphisms were incorrectly predicted to be deleterious based on conservation in the three known P gene sequences (human, pig, mouse). To achieve 82% sensitivity for predicting OCA1-associated tyrosinase variants, cutoff scores for AA conservation would classify 50% of codons as conserved, with resulting 50% specificity, 100% Pos Predictive Value (PPV), and only 9% Neg Predictive Value (NPV). For predicting OCA2-associated P gene variants, cutoff scores to achieve 85% sensitivity would classify 73% of codons as conserved, with resulting 50% specificity, 91% PPV, and 38% NPV.

CONCLUSIONS: 1) Adequate databases of sequences, mutations, and polymorphisms are needed to confirm the validity of predictions based on evolutionary AA conservation. 2) >80% prediction of deleterious mutations is possible. 3) More P Gene sequences are needed to validate evolutionary predictions.

A simulation study comparing three methods for individual admixture estimation. *V.L. Martinez-Marignac¹, M.R. Vaughn¹, I. Halder², M.D. Shriver², E.J. Parra¹.* 1) Department of Anthropology , University of Toronto, Mississauga, Ontario, Canada; 2) Department of Anthropology, Pennsylvania State University, University Park, USA.

Population-based association studies are a promising approach for mapping genes involved in complex traits and diseases. However, these studies are prone to false positive results due to population structure. One potential source of population structure is admixture stratification (e.g. variation of individual ancestry between individuals in an admixed sample). Admixture stratification can have a profound effect in association studies in admixed populations, potentially leading to significant results for a substantial number of genetic markers that are not functionally related to the complex trait being studied. The problems outlined above can be eliminated by controlling for individual ancestry in the statistical analysis. A number of software programs are available that implement different strategies to estimate individual ancestry in admixed populations. However, there has not been a systematic evaluation of the relative merits of these programs. In this study, we simulated different admixture scenarios and evaluated three widely used programs to estimate individual ancestry: STRUCTURE, ADMIXMAP and a maximum likelihood method (ML, Chakraborty 1986). We created, by simulation, admixed samples with different ancestral contributions and different levels of admixture stratification. The programs STRUCTURE, ADMIXMAP and ML were run on the simulated population data to determine the ability of each program to correctly identify individual admixture and population stratification under the alternative admixture scenarios. We explored the effect of factors such as the number of parental populations, the number of informative markers, the availability of parental frequency data (only tested for STRUCTURE and ADMIXMAP) and the effect of misspecification of parental frequencies (only tested for ADMIXMAP).

Association Studies between the Angiotensin I-Converting Enzyme (ACE) gene Polymorphisms and Essential Hypertension in Koreans. *D.J. Shin¹, J. Kim¹, Y. Bae¹, E. Kim¹, J. Kim¹, H. Park², C. Park², S. Kim³, J. Lee³, Y. Jang², S.J.K. Yoon¹.* 1) Research Institute of Molecular Genetics, Catholic Research Institutes, Seoul, Republic of Korea; 2) Cardiovascular Genome Center, Yonsei University Medical Center, Seoul; 3) DNA Link, Inc, Seoul.

The gene encoding angiotensin I-converting enzyme (ACE) is one of the most important candidate genes in studies of the genetic susceptibility to blood pressure (BP) regulation and essential hypertension (EH). Although there are many reports demonstrating the association of ACE gene polymorphisms with EH in different ethnic populations, the results are still controversial. The aim of this study is to evaluate whether ACE variants are associated with an increased risk of EH in a sample of 916 unrelated Korean subjects, comprised of 458 sex- and age-matched normotensive controls and 408 hypertensive cases with clinically diagnosed hypertension. We examined six polymorphisms (A2400T, C2547T, ACE I/D, G14480C, A14519G and A22982G) in the ACE gene. We found that 2400AA and 2547TT homozygotes independently conferred an increased risk to high BP in hypertensive females only ($p=0.004$ and 0.013 , respectively). When the subjects were divided into two groups by onset age, the frequencies of 2400AA, 2547TT, II and 14519AA genotypes were significantly associated with BP in the females aged 55 years ($p=0.001$, 0.005 , 0.026 and 0.026 , respectively). Conversely, in the older female subjects aged 55 years and in males, neither systolic nor diastolic BP was not statistically significant among the genotypes. In the haplotype-based analyses, combining all six ACE variants, we found that only one set of haplotype (ATICAA) was positively associated with hypertension (OR 1.244; 95% CI 1.008-1.535; $p=0.042$) in the younger subjects. Additionally, the ACE gene haplotype structure of the hypertensives was more heterogeneous than that of the normotensives. Our results demonstrated that ACE gene haplotype was associated with EH and might interact positively with 2400A and 2547T alleles of ACE gene in younger Korean females aged 55 years. Therefore, further study in this field will have a close clinical relevance to EH and cardiovascular disease..

Establishment of Taiwan Han Chinese Cell and Gene bank: Comparing SNP profiles in MHC region with Caucasians. *W.H. Pan¹, C.S.J. Fann¹, J.Y. Wu¹, S.I. Hung¹, Y.T. Hung², Y.J. Chen¹, C.L. Hsu¹, C.J. Liao¹, Y.T. Chen¹.*
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The purpose is several folds: (1) to preserve Taiwanese genetic materials, (2) to document their genetic variation, and (3) to serve as controls for disease gene mapping/association studies. 98 % of our population are composed of Chinese migrated from Fukien and Guandong provinces of China with the remaining 2 % of aborigines. We adopted a stratified, 3-staged, probability clustering sampling scheme. 329 non-aboriginal townships or city districts as primary sampling units (PSU) were grouped into 6 strata. Two to 8 PSUs were drawn in each stratum, proportional to its size. Then, 6 to 24 villages or city blocks were selected within each PSU. Within each village, included were 25 to 42 people aged 20 and older whose parents and grandparents were Han Chinese. This scheme was designed such that the proportion of samples in each stratum represents the distribution of the entire population and there were 278 subjects in each of the 6 age groups (20-, 30-, 40-, 50-, 60-, 70-) in men and in women, respectively. Plasma/DNA/lymphocytes were banked; blood chemistry, blood pressure, peak flow, and anthropometric parameters were measured; and questionnaire on ethnicity, disease history, life styles, and short portable mental status was administered. EBV-transformed lymphoblastoid cell lines were established. We completed the study in 3439 people. 94 individuals were randomly selected for SNP genotyping using MALTI-TOF mass spectrometry in the major histocompatibility complex (MHC) region (6p21.3). In 191 SNPs for which allele frequencies from 136 founders of CEPH families are available for comparison, 121 were significantly different ($p < 0.05$). Among these, 24 SNPs of CEPH families were not polymorphic in our population. For the remaining 70, allele frequencies were not statistically different. We conclude that SNP profiles in the MHC region differ considerably between Taiwan Han Chinese and CEPH families. This cell and gene bank is a powerful resource for studying genetic variation and disease association.

ALFRED The ALlele FREquency Database an update. *H. Rajeevan¹, K.-H. Cheung^{1,2}, S. Stein¹, H. Deng¹, J.R. Kidd¹, A.J. Pakstis¹, D. Chudnov², P.L. Miller², K.K. Kidd¹.* 1) Genetics, Yale U. Sch. Medicine, New Haven, CT 06520-8005 USA; 2) Medical Informatics, Yale U. Sch. Medicine, New Haven, CT.

ALFRED (<http://alfred.med.yale.edu>) is an actively curated database designed to make allele frequency data on anthropologically defined human population samples readily available to the scientific community. All data in ALFRED is considered to be in the public domain and available for use in research and teaching. All polymorphisms and most populations in ALFRED are linked to various external resources. The evolution of ALFRED has moved mainly in three directions: (1) a new GIS-based map interface to display population data on a map, (2) implementation of a new interactive data retrieval tool and synonyms/alternate names for ALFRED primary names, and (3) expansion of the database by increasing the quantity, quality, and accessibility of data in ALFRED.

The prototype SVG-based map interface will be replaced by a mature and stable product--GIS based map interface and analysis tools. This map interface will dynamically display all ALFRED populations and enable a user to make selections from the map based on geographic locations and then follow the links for population information or allele frequency tables. The new interactive data retrieval tool allows users to perform 2 types of searches: (1) seek entries in ALFRED, (2) seek frequency tables using gene and population names which will retrieve an updatable matrix of polymorphism x population indicating the presence or absence of frequency tables for each combination. ALFRED is updated daily and it currently has information on 987 polymorphic sites typed on at least one population sample and 358 populations typed for at least one polymorphism for a total of 21,010 frequency tables (one population typed for one site). Helpful search options and web viewing enhancements are also available from the web interface. For example, the list of publications underlying the data can now be searched by the name of any author and the allele frequency data linked to that particular publication can be quickly retrieved. ALFRED is supported by a grant from U.S. NSF BCS0096588.

Characterization of SNPs in human promoter sequences using dbQSNP, an SSCP-based SNP-

finding/quantification system. *T. Tahira*¹, *Y. Kukita*¹, *K. Higasa*¹, *Y. Suzuki*², *S. Sugano*², *K. Hayashi*¹. 1) Res Ctr Genetic Info, Med Inst Bioreg, Kyushu Univ, Fukuoka, Japan; 2) Dept Med Genome Sci, Grad School Frontier Sci, Univ Tokyo, Tokyo, Japan.

SNPs in promoter regions may potentially modify gene expression level and yield phenotypic diversity of individuals, i. e. disease susceptibility. We characterized SNPs in 1.2 kb genomic regions proximal to transcription start sites (TSS) at a large-scale using dbQSNP, a laboratory information management system that assisted SNP finding/allele frequency estimation by concerted analysis of SSCP and sequencing. SNPs found in this system were highly reliable, since most of SNPs were validated by two independent methods. We have screened SNPs in 8 Japanese individuals and collected 4969 SNPs in putative promoter regions of 3851 genes. Allele frequencies of 3134 SNPs were determined by quantifying SSCP peaks of two pools (Japanese and Caucasian) after compensating for unequal peak heights in heterozygotes, and 2138 of them were assigned as frequent SNPs (SNPs of minor allele frequency >10% in Japanese). To examine the efficiencies of our SNP-discovery pipeline, we Blast-searched the genomic regions targeted by us against external SNP databases. Approximately 34% of our SNPs (18% of our frequent SNPs) in these regions were new (not in dbSNP, build 121). Only one eighth of our SNPs was registered in JSNP. We compared our allele frequency values with those in JSNP using SNPs common in the two databases. The two values were in excellent correlation ($R^2 = 0.85$) although the subjects were independently collected in two distant areas in Japan, indicating Japanese as well mixed population. On the other hand, allele frequencies were strikingly different between Japanese and Caucasian ($R^2 = 0.40$). Some of the SNPs revealed high F_{st} values (>0.5), possibly due to differential selection between the two populations. All the above data were made accessible through dbQSNP Public.

Polymorphism in both promoters of Hepatocyte Nuclear Factor 4-alpha are associated with Type 2 Diabetes in the Amish. *C.M. Damcott¹, N. Hoppman¹, S.H. Ott¹, L.J. Reinhart¹, J. Wang¹, J.R. O'Connell¹, B.D. Mitchell¹, A.R. Shuldiner^{1,2}.* 1) Division of Endocrinology, Diabetes, and Nutrition, University of Maryland School of Medicine, Baltimore, MD; 2) Geriatric Research and Education Clinical Center (GRECC), Veterans Administration Medical Center, Baltimore, MD.

Hepatocyte nuclear factor 4-alpha (HNF4A) is a transcription factor that regulates expression of genes involved in glucose metabolism and insulin action. HNF4A is located on chromosome 20q13 in a region of overlapping linkage to type 2 diabetes (T2DM) in several Caucasian and Asian populations. Recently, two groups independently identified SNPs in an alternate upstream promoter (P2) of HNF4A that were associated with T2DM and explained a large portion of the reported linkage in their Ashkenazi Jewish and Finnish populations. We genotyped haplotype-tagging SNPs (htSNPs) across the two promoter regions and the coding region of HNF4A in individuals with T2DM (n = 137), impaired glucose tolerance (IGT) (n = 139), and normal glucose tolerance (NGT) (n = 342) from the Amish Family Diabetes Study (AFDS) to test for association with T2DM and related traits. We observed a significant association between the rs2425640 SNP in the P1 promoter region and T2DM (OR = 1.60; p = 0.03) and a borderline association between the rs1884614 SNP, an htSNP representing the haplotype block that includes the P2 promoter, and both T2DM (OR = 1.40; p = 0.09) and the combined T2DM/IGT trait (OR = 1.35; p = 0.07). We subsequently genotyped rs1884614 and rs2425640 in an expanded set of 821 nondiabetic AFDS subjects and found association between rs1884614 and glucose area under the curve during an oral glucose tolerance test (additive model: p = 0.022; dominant model: p = 0.010). The results of this study provide evidence that variants in both the P1 and P2 promoters of HNF4A increase risk for typical T2DM.

Linkage disequilibrium in the region of the putative diabetes gene, calpain-10 (*CAPN10*). *L. del Bosque Plata*¹, *M.G. Hayes*², *N.J. Cox*^{2,3}, *G.I. Bell*^{1,2,3}. 1) Biochemistry & Molecular Biology, Univ Chicago, Chicago, IL; 2) Human Genetics, Univ Chicago, Chicago, IL; 3) Medicine, Univ Chicago, Chicago, IL.

Patterns of linkage disequilibrium (LD) depend on a number of factors including population size and structure, founder effects and admixture, as well as gene-specific factors such as selection and rates of mutation and recombination. Recent studies have shown considerable heterogeneity in LD between regions and among populations suggesting it is important to characterize these underlying patterns in order to effectively use LD for fine-scale mapping of disease genes. We present here LD maps for several populations at calpain-10 (*CAPN10*) a gene associated with altered risk of type 2 diabetes mellitus. We analyzed 42 SNPs spanning a 483,498 bp region in 510 normal individuals of different ancestry: African American 50; European 62; Chinese (Southern Han) 49; Japanese 28; Mexican (mestizo) 52; and Amerindian 269 (Nahua 41, Otomie 57, Totonaca 43, Mazatecan 74, Huastecan 28 and Zapotecan 26). We used the measures D , r^2 , Collins and Mortons LD Units, and the population genetic parameter for recombination ρ (ρ), to assess LD differences between populations. One thousand bootstrap samples were collected to obtain the variance in estimates of D and the Wilcoxon signed-ranks test was used to test for significant differences in between populations. We found that *CAPN10* is located within a block of LD conserved across all populations although the level of LD varied between the populations. Across the entire region, we found lower levels of LD in African-Americans than in European and Asian populations. The Mexican group had more extensive LD than Europeans. The Amerindians had the most extensive LD with LD varying among the populations, both in extent and pattern, consistent with the complex and different patterns of demographic history in the different groups. We also found differences in population recombination rate ρ , among the different groups that we studied implying major differences in population history in this region.

Mitochondrial DNA variability in Koreans and Mongolians. *A.V. Lunkina*¹, *M.V. Derenko*¹, *T. Grzybowski*², *B.A. Malyarchuk*¹, *I.A. Zakharov*³, *D. Miscicka-Sliwka*², *D.T. Tsedev*⁴, *K.S. Park*⁵, *Y.M. Cho*⁵, *H.K. Lee*⁵, *Ch.H. Chu*⁶. 1) Genetics Department, Institute of Biological Problems of the North, Magadan, Russian Federation; 2) The Ludwik Rydygier Medical University in Bydgoszcz, Bydgoszcz, Poland; 3) Vavilov Institute of General Genetics, Moscow, Russia; 4) Institute of Biological Sciences, Mongolian Academy of Sciences, Ulaanbaatar, Mongolia; 5) Seoul National University, College of Medicine, Seoul, Korea; 6) Department of History, Kangwon National University, Chunchon, Korea.

Based on comprehensive mtDNA (HVR1/HVR2 sequencing combined with RFLP typing of informative coding region polymorphisms) analysis the gene pools of Koreans (N=164) and Mongolians (N=48) were characterized in details. It was shown that their gene pools are represented by a common set of East Eurasian mtDNA haplogroups (M7c, M8a, C, D4, G1a, G2, A, B4, B5, F1a, F1b, N9a). In addition to aforementioned haplogroups, haplogroups M7a, M7b, M9a, G3, F1c, D5, and Y were identified in Koreans, and haplogroups M10 and Z in Mongolians. Caucasoid-specific component represented by West Eurasian mtDNA haplogroups K, U4 and N1a was found only in Mongolians with a frequency of 10.4 per cent. The phylogenetic and statistical analyses results based on mtDNA variability data in South Siberian, Central and East Asian populations demonstrate the considerable degree of inter-population differentiation which is determined both by geographical and linguistical factors. Analysis of pairwise F_{ST} -distances has demonstrate the considerable genetic similarity between Koreans and Northern Han populations, which are, in turn, clearly differentiated from Southern Han populations. Mongolians occupy the intermediate position between South Siberian and Central/East Asian groups. This study was partly supported by Russian Foundation for Basic Research (grant N. 04-04-48746).

Genetic variation in "reproductive switch" and aging in *Drosophila*. *N. Abedi, M. Beikpoor, A. Zamiri, M. Zeinali, S.M. Hosseini-Mazinani, J. Koochmeshgi.* Research Institute for Life, Tehran, Iran.

Exploring evolutionary pressures underlying the relationship between reproductive parameters (e.g.: age at maturity) and aging is one of the key themes in aging research. We propose that "reproductive switch" is at the heart of this relationship. In a basic model, we divide the life history of an organism into two phases: pre-reproductive and reproductive. In the pre-reproductive phase, life is geared towards optimal health and in the reproductive phase towards optimal reproduction, though some aspects of organism's life in this phase may be detrimental to health in the long-term. This concept is consistent with the notion of antagonistic pleiotropy. The later an organism throws the "reproductive switch" and enters the reproductive phase, the longer it lives. We propose that there is an inherent genetic variation in the timing of reproductive switch. In some individuals in the population, this switch is thrown sooner, i.e. with less food intake and in some individuals later, i.e. with more food intake. This variation allows the population as a whole to adapt to varying levels of food availability. Based on this concept, we try to understand *Drosophila* breeding experiments that aim at life extension. We propose that overcrowding at the larval stage is the main factor in these experiments, bringing out the inherent variation in the timing of the genetic switch: Under overcrowded conditions, those larvae needing less food to throw the switch enter the reproductive phase of adult fly sooner but those needing more food take longer and consequently live longer. While under normal culture conditions of abundant food, all larvae reach the reproductive phase of adult fly more or less at the same time. Currently, we are focusing on a series of experiments based on this concept and investigating the possible effects of overcrowding on age at death, pattern of reproduction, and body size in individuals of populations of *Drosophila*.

Evidence of Homoplasmy in Insulin Degrading Enzyme (IDE) and the Implications for Studies of Association.

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We used 19 SNPs in 265 controls and 300 cases perform cladistic analyses of phenotypic association. Haplotypes were estimated using the software PHASE. A set of 95% plausible haplotype trees was estimated using statistical parsimony in TCS. The tree was inspected for recombination and recombinant haplotypes were removed. Haplotype categories were defined by a nesting algorithm. Association between Alzheimers disease and haplotypes were investigated using a series of nested contingency analyses. From the PHASE results we inferred the existence of 54 haplotypes in our sample population. Two statistically significant recombination events were detected in the tree ($p=0.05$ and 0.01). The tree also shows evidence of homoplasmy in most of these SNPs. No significant permutational chi-square values were detected in the nested contingency analyses. The results of the cladistic analyses of phenotypic association are consistent with the single SNP analyses of this population. However, the evidence of homoplasmy has some implications regarding the non-replication between samples, which is often observed between studies investigating this region. Sites showing homoplasmy are not ideal as single-site markers of association because identity by state does not reflect identity by descent. Under such conditions patterns of linkage disequilibrium can be complex, possibly resulting in increased rates of type II error. Thus, the use of homoplasious sites as single-site markers of association can confound traditional association studies and may contribute to our inability to replicate results between sample populations.

Characterization of melanocortin 1 receptor variants in Asians and Oceanians. *K. Nakayama*¹, *A. Soemantri*², *F. Jin*³, *S. Harihara*¹, *T. Ishida*¹. 1) Department of Biological Sciences, The University of Tokyo, Tokyo, Japan; 2) Diponegoro University, Semarang, The Republic of Indonesia; 3) The Chinese Academy of Sciences, Beijing, The Peoples Republic of China.

Melanocortin 1 receptor (MC1R), a Gs protein coupled receptor, plays a pivotal role in regulation of eumelanin and pheomelanin synthesis in melanocytes. The MC1R gene (*MC1R*) is known to be polymorphic among Europeans where several nonsynonymous variants of the *MC1R* have been associated with fair skin color and red hair. Such nonsynonymous variants show dramatic decrease in intracellular cAMP production following -melanocyte stimulating hormone (MSH) treatment. We previously identified nine nonsynonymous variants, Arg67Gln, Val92Met, Ile120Thr, Phe147, Arg151Cys, Thr157Ile, Pro159Thr, Arg163Gln and Ala166Gly, of the *MC1R* in 30 Asian and Oceanian populations; however, functional consequences of these variants are still unknown. To characterize the potential of intracellular cAMP production in these nine nonsynonymous variants, we measured activity of MC1R by using transient expression in COS-7 cells and enzymoimmunological assay. Among these nine MC1R variants, Phe147, Arg151Cys, Ile157Thr and Pro159Thr variants, which were identified among high latitude East Asians, showed dramatic decrease in cAMP production. These four variants were located in the second intracellular loop of MC1R that is important to G protein coupling; therefore, the decrease in cAMP production is expected to be caused by disruption of G protein coupling rather than insufficient -MSH binding. It is thus conceivable that the rare presence of individuals with blond hair and fair skin color in high latitude East Asians. Arg67Gln, Val92Met, Ile120Thr, Arg163Gln and Ala166Gly variant slightly lowered cAMP production compared with wild type MC1R, however, there were no substantial effects on dose-dependent response to -MSH stimulation. The present results support participation of nonsynonymous variants on the *MC1R* in variation in pigmentation phenotypes of Asians.

Recent Primate and ongoing human evolution of the CMT1A-REPs. *T. Ohyama*¹, *M. Khajavi*¹, *M. Hurles*², *J.R. Lupski*¹. 1) Dept. of Mol. & Human Genetics, Baylor College of Medicine, Houston, TX; 2) The Wellcome Trust Sanger Institute, Cambridge, UK.

Structural features of the human genome can result in a genome architecture leading to susceptibility for DNA rearrangements associated with genomic disorders such as Charcot-Marie-Tooth disease type 1A (CMT1A) and hereditary neuropathy with liability to pressure palsies (HNPP). This genome architecture consisting of region specific low copy repeats (LCR) provides homologous sequence substrates for nonallelic homologous recombination (NAHR) that result in DNA rearrangements. Previous studies indicate that LCRs evolved through segmental duplications during primate speciation and likely still undergo evolutionary changes potentially making the human genome more susceptible to genomic rearrangements. Here, we report the results of screening 96 DNAs from 48 world populations for CMT1A-REP LCRs using the HGDP-CEPH Human Genome Diversity Panel (www.cephb.fr/HGDP-CEPH-Panel/). In all analyzed populations we identified two CMT1A-REPs, but in the samples from Central Africa, we observed the presence of different sized bands. In order to examine the evolution of CMT1A-REPs, we identified and sequenced nonhuman primate BAC clones which contain the entire proximal CMT1A-REP in chimpanzee and distal CMT1A-REP in chimpanzee and gorilla, respectively. We concluded that CMT1A-REPs are dynamically evolving and the pattern and rate of nucleotide substitution potentially play a critical role in the continuous genome evolution.

Genetic Structure of Indian Populations. *N. Rosenberg*¹, *C. Gonzalez*², *L. Nino-Rosales*⁴, *V. Ninis*⁴, *P. Das*⁴, *L. Molinari*³, *G. Zapata*³, *J. Weber*⁵, *J. Belmont*³, *P. Patel*^{2,3}. 1) Dept Molec Comp Biol, Univ Southern California, Los Angeles, CA; 2) Institute for Genetic Medicine, Univ Southern California, Los Angeles, CA; 3) Dept Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 4) Dept Neurology, Baylor College of Medicine, Houston, TX; 5) Marshfield Clinic Research Foundation, Marshfield, WI.

There is a growing recognition of the importance of assessing genetic variation amongst various human populations, not only for understanding the origins and evolution of humans, but also because of its relevance to the genetic dissection of complex disease, and response to environmental agents, such as drugs. Despite the large diversity of peoples within India, populations from India have been largely underutilized in studies of genetic variation. We have begun a study of the structure of populations whose mother tongue is one of 15 major Indian languages by conducting a genome scan (Marshfield Panel 13) in 24 unrelated individuals from each group. Within one group that speaks Gujarati, we are analyzing 24 unrelated individuals from each of six villages in Gujarat State that practice endogamy to determine if such groups represent genetic isolates. Using these data, in conjunction with worldwide data on the same markers (Rosenberg et al., *Science* 298:2381-2385, 2002), we will determine the pattern of population structure and admixture within India, and we will analyze the relationships of groups from India to those from other parts of the world. The analysis will identify markers of particular informativeness for ancestry inference within India, and will be more broadly applicable to genetic mapping in individuals of Indian ancestry.

Mitochondrial polymorphism in prehistoric and present-day Andean populations of Chile: Signatures of chronological evolution and population size changes. *F. Rothhammer*¹, *S.A. Barton*², *R. Chakraborty*³. 1) Human Genetics Program, Faculty of Medicine, University of Chile, Santiago, Santiago 7, Chile; 2) Human Genetic Center, Univ. Texas School of Public Health, Houston, TX; 3) Center for Genome Information, Dept. Environmental Health, Univ. Cincinnati, Cincinnati, OH.

To investigate whether or not the prehistoric populations of Chile represent the ancestors of the present-day native populations of Chile, this research used the mitochondrial haplogroup and control region sequence data recently published from these populations under the framework of analysis of longitudinal population-based data. Data analyzed consists of haplotype information from 61 prehistoric mtDNA samples (of age ranging from 3,900 to 500 yrs old), and 172 contemporary individuals. The results indicate that the temporal changes of mtDNA haplogroup diversity and sequence mismatch distributions between samples of contemporary and prehistoric populations are consistent with the hypothesis of chronological evolution. This observation is also confirmed by phylogenetic reconstruction of control region sequence evolution of 30 prehistoric samples, and 43 contemporary samples. During the period of 3,900 years of evolution, the haplogroup A showed evidence of declining frequencies over time, with conjoint increase of the frequency of haplogroup B. Analysis of data under the framework of temporal evolution suggests considerable changes of effective population size over time (i.e., reduction of effective size by at least one-third, over the last 500 years of evolution), with evolutionary rates of divergence in the mtDNA genome remaining virtually unchanged over time. Low frequency of haplogroup B in prehistoric populations of Chile has also relevance for natural history of disease occurrences in this part of this region, particularly for diseases that are influenced by mitochondrial mutations. (Data analysis of this research is supported by NIH grant GM 41399 to RC).

A robust test of selective neutrality of SNPs in different pathways of environmentally relevant genes. X. Sheng, R. Chakraborty. Ctr Genome Information, Univ Cincinnati, Cincinnati, OH.

Resequencing efforts of the NIEHS-sponsored environmental genome project (EGP) have generated a public domain database of single nucleotide polymorphism (SNP) sites residing on a large number of environmentally relevant genes of different pathways (<http://egp.gs.washington.edu>). Genotypes on these SNP sites have been scored on individuals of different anthropological origin, making this database subject to population substructure effects. Consequently, to examine whether or not these SNP sites are subject to forces of natural selection, analytic methods have to be robust against the impact of population substructure effects. Under the assumption that the SNP sites follow the infinite site model of mutations, the contribution to relative heterozygosity, plotted against the gene frequencies of alleles, provide such a robust test. Extracting polymorphism data on a total of 10,152 SNPs on 92 genes belonging to 7 pathways (apoptosis; cell cycle; base excision repair, BER; nucleotide excision repair, NER; transcription coupled repair, TCR; double strand break repair, DSB; and mismatch repair, MMR) from the EGP database, we conducted tests of selective neutrality of these SNPs. Our results indicate that SNPs on three pathways have mild signatures of natural selection (deleterious selection effects seen for 2,601 SNPs on 26 genes of the BER pathway, and for 1,030 SNPs of 4 genes of the MMR pathway, and overdominant selection effect for 1,397 SNPs on 12 genes of the DSB pathway). These results are not always consistent with alternative tests already performed on this dataset, and in part this discordance is due to the effect of underlying population substructure present in the database. Considering that our test procedure (based on relative heterozygosity contributed by alleles of different gene frequency classes) is invariant under population substructure, we conclude that this robust test provides indications of existence of both overdominant and deleterious mutations in maintaining polymorphisms at SNP sites residing on functional genes of several pathways. (Research supported by the NIH grant GM 41399 to RC).

Accelerated evolution of the PACAP precursor gene during human origin. *B. Su*^{1,2}, *YQ. Wang*¹, *YP. Qian*², *S. Yang*¹, *H. Shi*¹, *CH. Liao*¹, *HK. Zheng*³, *J. Wang*³, *A. Lin*⁴, *L. Cavalli-Sforza*⁴, *P. Underhill*⁴, *R. Chakraborty*², *L. Jin*^{2,5}.
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Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide abundantly expressed in the central nervous system, and involved in regulating neurogenesis and neuronal signal transduction. The amino acid sequence of PACAP is extremely conserved across vertebrate species, indicating a strong functional constraint during the course of evolution. However, through comparative sequencing, we demonstrated that the PACAP precursor gene underwent an accelerated evolution in the human lineage since the divergence from chimpanzees, and the amino acid substitution rate in humans is at least seven times faster than in other mammal species resulting from strong Darwinian positive selection. Eleven human-specific amino acid changes were identified in the PACAP precursors which are conserved from murine to African apes. Protein structural analysis suggested that a putative novel neuropeptide might have originated during human evolution and functioned in the human brain. Our data suggested that the PACAP precursor gene underwent adaptive changes during human origin and may contribute to the formation of human cognition.

Genetic evidence supports demic diffusion of Han culture. *B. Wen¹, H. Li¹, D. Lu¹, X. Song¹, F. Zhang¹, Y. He¹, F. Li¹, Y. Gao¹, X. Mao¹, L. Zhang¹, J. Qian¹, J. Tan¹, J. Jin¹, W. Huang², R. Deka³, B. Su^{1,3,4}, R. Chakraborty³, L. Jin^{1,3}.*

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The spread of culture and language in human populations is explained by two alternative models: the demic diffusion model (DDM) which involves massive movement of people, and the culture diffusion model (CDM) that refers to the occurrence of mainly cultural impact between populations with limited genetic exchanges. The mechanism of the peopling of Europe has long been debated on whether the diffuse of agriculture and language from Near East was concomitant with substantial movement of farmers. The Han people which share the same culture and language exceeds 1.16 billion (2000 census), and is by far the largest ethnic group in the world. To understand the expansion process of Han culture is thus of great interests to researchers in many fields. Here we show, by systematically analyzing Y chromosome and mitochondrial DNA (mtDNA) variations in the extant Han populations, the pattern of the southward expansion of Han culture is consistent with DDM, and more interestingly, males played a larger role in this expansion.

Selective trends in 22 bitter-taste receptor genes. *S. Wooding*¹, *U.-k. Kim*², *L.B. Jorde*¹, *D. Drayna*². 1) Dept. of Human Genetics, Univ. of Utah, Salt Lake City, UT; 2) NIDCD, NIH, Rockville, MD.

Bitter-taste perception in humans is mediated by a small family of G-protein coupled receptors that reside on the surface of taste buds, where they are exposed to potential ligands as they pass over the tongue. Anecdotal evidence has long suggested that bitter-taste perception played an important role in the course of human evolution by allowing humans to detect and avoid the many bitter toxins produced by plants as a means of defense against herbivores. Patterns of variation in one bitter-taste receptor gene, T2R38, are consistent with the view that balancing natural selection has fostered high levels of diversity. To determine whether other bitter taste receptor genes have been under similar selective pressures, we resequenced the complete coding regions of 21 bitter taste receptor genes in 60 humans from Africa (n = 31), Asia (n = 10), Europe (n = 9), and North America (n = 10). Tajima's D test for selective neutrality revealed that when tested individually, no D value departed significantly from theoretical expectation; however, the mean observed D value (= -0.36) was significantly greater than theoretical expectation under the assumption of population growth ($p < 0.01$) and was also significantly greater than the mean in a sample of more than 1600 other genes ($p < 0.01$). Similarly, while no individual FST value was significantly greater than expected, the mean observed FST was significantly greater than a simulated expectation ($p < 0.01$) and was also significantly greater than the mean in a sample of more than 25,000 SNPs. These findings suggest that human populations are, on average, more different with respect to bitter-taste receptor genes than they are with respect to other regions of the genome, a pattern best explained by the effects of recent local adaptation.

Rapid evolution of two Pexin-domain-containing proteins in Primates; implications for Human disease. *G.J. Wyckoff, A. Bulavtseva, J.E. Paschall, M. Yang.* Div Molec Biol & Biochemistry, Univ Missouri, Kansas City, Kansas City, MO.

We undertook to examine the evolution of matrix metalloproteinase genes within primates. Hemopexin (HPX) and matrix metalloproteinase 12 (MMP12) are both members of the metalloproteinase family of proteins, which bind free metal ions (e.g., copper or iron) in the body. Mutation or dysfunction in matrix metalloproteinase genes may be important causal factors in human disease, as they protect against oxidative stress. Absence of MMP12 in model organisms leads to greater rates of emphysema, whereas HPX is a potential biomarker for Alzheimers Disease and may be causally involved with the disease state. In examining 16,000 human-mouse gene orthologs and more than 200,000 protein motifs, we determined that both HPX and MMP12 contain hemopexin-like repeat domains that are rapidly evolving and have Ka/Ks rates above 1.0: a hallmark of positive, Darwinian selection. The N-terminal Pexin-domain in HPX has a Ka/Ks ratio of 1.1 between humans and mice, compared with a gene-wide average of 0.4. The C-terminal Pexin-domain of MMP12, in contrast, has a Ka/Ks of 0.81, versus a gene-wide average of 0.5 for the mouse-human ortholog comparison. Analysis of fixed differences in this region shows that rare amino acid changes are abundant. Although there are many members of the metalloproteinase gene family, only HPX and MMP12 showed convincing evidence of Darwinian selection. Our further examination of human polymorphism in these genes suggests that Darwinian pressure has been active during the evolution of these genes in primates. Since HPX and MMP12 are demonstrably under selection, possibly due to their functional roles, we suggest that this evolutionary analysis may help to augment future research into diseases associated with oxidative stress.

Adaptive evolution of MrgX2, a human G protein coupled receptor involved in nociception. *S. Yang¹, Y. Liu¹, A. Lin³, L. Cavalli-Sforza³, B. Su^{1,2}.* 1) Kunming Institute of Zoology, the Chinese Academy of Science, Kunming, Yunnan, China; 2) Center for Genome Information, Department of Environmental Health, University of Cincinnati, Cincinnati, OH; 3) Department of Genetics, Stanford University, Stanford, CA.

MrgX2 is a G-protein coupled receptor which is specifically expressed in the sensory neurons of the peripheral nervous system and involved in nociception. We sequenced the coding region of the MrgX2 gene in world-wide human populations and seven representative nonhuman primate species. Our results indicated that MrgX2 evolved rapidly in primates, especially in the lineage leading to humans. We observed four fixed amino acid substitutions in humans that were caused by strong Darwinian positive selection, implying that MrgX2 has undergone adaptive changes during human evolution. By reconstructing the secondary structure of the human MrgX2, we demonstrated that three of the four human-specific amino acid substitutions are located in the extra-cellular domains, and may alter the interaction between MrgX2 and its ligand, consequently, contributing to the adaptive change of human nervous system during human origin.

Evolution of abcr proteins in vertebrates. *A.N. Yatsenko, W. Wiszniewski, M. Jamrich, J.R. Lupski.* Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

The *ABCR* (*ABCA4*) gene encodes a retinal-specific ATP-Binding Cassette transporter. Mutations of *ABCA4* are responsible for several macular degenerations. The protein appears to function as a flippase of all-*trans*-retinaldehyde (*atRAL*) and/or its derivatives across the membrane of outer segment (OS) disks, and is a potentially important element in recycling visual cycle metabolites. However, our understanding of the role of ABCR in the visual cycle is limited due to the lack of a direct functional assay. An evolutionary analysis of ABCR may aid in the identification of conserved elements, the preservation of which implies functional importance. To date, only human, murine, and bovine *ABCR* genes have been described. We have identified *abcr* genes from African (*X. laevis*) and Western (*S. tropicalis*) clawed frogs. Using sequences of the frog *abcrs*, annotated *T. rubripes* *abcr*, and mammalian ABCR proteins, we performed a comparative analysis and describe the evolutionary relationships between the vertebrate *abcrs*. We note that in addition to the transmembrane and ATP-binding domains, several segments are conserved in both intradiscal loop (IL) domains. Nonconserved segments were found in the IL and cytoplasmic linker (CL) domains. Our collective data support the current model that two distinct *abcr* progenitors (half-transporters) combined to form a full *abcr* progenitor in ancestral chordates. We speculate that evolutionary alterations may increase the retinoid metabolite recycling capacity of ABCR, and potentially improve dark adaptation.

Ethiopia: between Sub-Saharan Africa and Western Eurasia. *V. Yotova*¹, *A. Lovell*¹, *C. Moreau*¹, *S. Bourgeois*¹, *F-X. Xiao*¹, *D. Gehl*¹, *J. Bertranpetit*², *E. Schurr*³, *D. Labuda*^{1,4}. 1) Centre de Recherche, Hopital Sainte-Justine, Universite de Montreal, Montreal, Quebec, Canada; 2) Unitat de Biologia Evolutiva, Universitat Pompeu Fabra, Barcelona, Catalonia, Spain; 3) Centre for the Study of Host Resistance, McGill University, Montreal, Quebec, Canada; 4) Departement de Pediatrie, Universite de Montreal, Montreal, Quebec, Canada.

Situated at the gateway of the southern out-of-Africa route, Ethiopia has become central to population genetic studies investigating the out of Africa expansion of modern humans, as shown by Y chromosome and mtDNA studies. To gain a complementary perspective to the contribution of the maternal or paternal lineages, we have analyzed a sample of 72 chromosomes from Semitic speaking Amhara, Cushitic speaking Oromo and Ethiopian Jews with the X-linked 8kb *dys44* haplotype at Xp21, and compared these results with 826 chromosomes from Middle Eastern, African, Asian and European populations. We report that the distribution of alleles of a variable T_n repeat, and the spread of haplotypes containing Africa specific alleles, provides evidence of a genetic continuity from Sub-Saharan Africa to the Near East, and furthermore suggests that a bottleneck occurred in Ethiopia. Ethiopian genetic heterogeneity, most likely resulting from periods of admixture, can clearly be seen in principal component analysis of haplotype frequencies. The two largest ethnic groups of Ethiopia, the Amhara and Oromo, were not found to be statistically distinct, based on an exact test of haplotype frequencies, presumably due to widespread gene flow within and across the region. The Ethiopian Jews also appear as an admixed population, possibly of Jewish origin, though the current data does not allow confident conclusions to be drawn regarding their ancestry. However, evidence of a closer relationship between Ethiopian and Yemenite Jews is provided, likely a result of indirect gene flow between the two and/or neighboring populations.

Signature of a selective sweep at the human CYP3A locus. *K.R. Ahmadi¹, S. Tate¹, M.E. Weale¹, Z.Y. Xue², N. Soranzo¹, D.P. Yarnall², J.D. Briley², T.D. Spector³, N.K. Spurr², D.K. Burns², A.D. Roses², A.M. Saunders², D.B. Goldstein¹.* 1) Biology (Galton Labs), University College London, London, UK; 2) Genetics Research, GlaxoSmithKline, Research Triangle Park, North Carolina; 3) Twin Research & Genetic Epidemiology Unit, St Thomas Hospital, London, UK.

The members of the CYP3A subfamily are the most abundantly expressed cytochrome P450 enzymes in the liver and are responsible for the metabolism of around half of all prescribed drugs. Until now there have not been a systematic study into the role of selection in shaping the genetic diversity at this locus. Our aim was to study the pattern of variation at the CYP3A locus in the CEPH and the Japanese. We genotyped a total of 67 SNPs throughout the CYP3A cluster (1 SNP / 3.5 kb). In support of previous reports our results showed that in the CEPH no SNPs were found to have a frequency higher than 7% in almost 150 kb of this cluster. We also found a similar consistent pattern in the Japanese although in this population all but 3 of the variants had a minor allele frequency between 25 and 30% ($F_{st} \sim 0.2$). The pattern of LD in the two populations was remarkably similar with the cluster falling almost entirely into a single block of LD with the same 2 haplotypes making up 95% of the total haplotype diversity. We used the data from the HapMap to show that at least in the CEPH this remarkable lack of diversity over such a long stretch of the human genome is rare. We speculate that the most likely target for this selection is the CYP3A5*3 splice variant which is responsible for a significant reduction in functional CYP3A5 protein and has been previously associated with systolic BP (sBP) in African Americans. To look at the pattern of geographic variation for this allele, we genotyped the *3 allele in populations from Southern Italy, China, India, Ethiopia, Cameroon, the Pacific, and a total of 877 Caucasian female individuals from the adult twin registry in the UK with sBP and diastolic BP (dBP) measurements. Our results show a remarkable degree of differentiation for the ancestral allele with a global F_{st} value close to 0.3. We fail however to show a significant association between the *3 allele and sBP and dBP in Caucasians.

Positive selection in MAOA gene is human exclusive: determination of the putative amino acid change selected in the human lineage. *A.M. Andres^{1,4}, M. Soldevila¹, A. Navarro¹, K.K. Kidd², B. Oliva³, J. Bertranpetit¹*. 1) Unitat de Biologia Evolutiva, Facultat de Ciències de la Salut i de la Vida, Universitat Pompeu Fabra (UPF), Barcelona, Spain; 2) Department of Genetics, Yale University School of Medicine, New Haven, USA; 3) Laboratori de Bioinformàtica Estructural (GRIB), Universitat Pompeu Fabra, Barcelona, Spain; 4) Current address: Molecular Biology and Genetics, Cornell University, Ithaca, NY, USA.

Monoamine oxidase A (MAOA) is the X-linked gene responsible for deamination and subsequent degradation of several neurotransmitters and other amines. Among other activities, the gene has been shown to play a role in locomotion, circadian rhythm and pain sensitivity, and to have a critical influence on behavior and cognition. Previous studies reported a non-neutral evolution of the gene, due to positive selection in the human lineage. To determine whether this selection was human-exclusive or shared with other species, we performed a population genetic analysis of the pattern of nucleotide variation in non-human species, including bonobo, chimpanzee, gorilla, and orangutan. Footprints of positive selection were absent in all but the human lineage, suggesting that positive selection has been recent and unique to humans. To determine which human-unique genetic changes could have been responsible for the differential evolution, the whole coding region of the gene was compared between human, chimpanzee and gorilla. Only one human exclusive non-conservative change is present in the gene: Glu151Lys. This human substitution affects protein dimerization according to a 3D structural model that predicts a non-negligible functional shift. It is the only candidate position at present to have been selected to fixation in humans during an episode of positive selection. Divergence analysis among species showed that, even under positive selection in the human lineage, the MAOA gene did not experience accelerated evolution in any of the analyzed lineages.

Segmental duplications define regions of chromosome and gene evolution in mammal genomes. *L. Armengol¹, J. Cheung², T. Marquès-Bonet³, A. Navarro³, S.W. Scherer², X. Estivill¹.* 1) Center for Genomic Regulation, Barcelona, CATALUNYA; 2) Department of Genetics and Genomics, Hospital for Sick Children, Toronto, Canada; 3) Pompeu Fabra University, Barcelona, CATALUNYA.

Segmental duplications (SDs) are known to have a role in gene evolution. Both generating new copies of genes that can accumulate mutations without being constrained by evolution and by generating totally new genes by fusion of different transcripts. A role in chromosomal evolution has also been suggested since they have been found to be enriched in human/mouse synteny breakpoints. We decided to confirm this implication of SDs in synteny breakpoints and we used two evolutionary close organisms whose genomic sequences are available: rat and mouse. Latest alignments between mouse and rat genome sequences were obtained from the UCSC. Over 1.5 million anchors were chained into 1953 syntenic clusters longer than 250 kb (Manhattan distance). Those were further grouped into higher order structures and resulted in a total of 102 synteny blocks. These synteny blocks defined 82 and 81 regions of break of synteny (BOS) respectively in the mouse and rat genomes. GC content, repeat composition, gene content, and presence of SDs was assessed in the whole no-block regions and in a region of +/-25 kb around the boundaries of the breaks of synteny. No significant differences in GC content or in repeat composition were observed. Gene content analysis revealed that breakpoint regions are gene rich regions, while no differences are observed if we compare no-block and syntenic regions. SDs were present in the majority of synteny break regions both in the mouse and in the rat genomes. When compared to a random distribution of breakpoints along the chromosomes, the association was very significant. Rates of evolution (Ka/Ks ratio) were also calculated, and were found to be higher for genes in no-block regions than for the rest of genes in the genome. This difference was even higher when taking into account genes located on SDs. The confirmation of the presence of SDs in regions of BOS in species from a different evolutionary lineage reinforces the view of a role of SDs as hotspots for chromosomal evolution.

Y-chromosomal SNP-STRs variation in Russian populations of Eastern Europe. *M.V. Derenko¹, B.A.*

Malyarchuk¹, M. Wozniak², T. Grzybowski², J. Czarny², D. Miscicka-Sliwka². 1) Institute of Biological Problems of the North, Magadan, Russia; 2) The Ludwik Rydygier Medical University in Bydgoszcz, Forensic Medicine Institute, Bydgoszcz, Poland.

We analyzed 12 Y-chromosomal SNP markers (RPS4Y, SRY-8299, M89, 12f2, M9, M20, 92R7, SRY-10831, DYS199, Tat, LLY22g, YAP at DYS287) and 10 microsatellite markers (DYS19, DYS385, DYS389, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439) in 338 males from 8 Russian populations of Eastern Europe. SNP analysis demonstrates that Russian gene pool is very close to central-eastern European populations. In AMOVA analysis using SNP markers, 5 per cent of the Y-chromosome variation was found between Russian populations. The MDS analysis based on Y haplogroup FST values revealed that the strongest division was between some northern Russian populations (Pskov and Pomor Russians) together with all Uralic- and Baltic-speaking populations and the remaining Russian populations clustered together with Slavonic-speaking groups (such as Poles, Ukrainians, Belorussians). Similarity of northern Russians with Uralic- and Baltic-speaking populations of Eastern Europe may be explained by increased frequency of haplogroup N3 (more than 35 per cent) found in these populations. Y-STR analysis within haplogroup N3 allowed determining a ratio of Uralic and Baltic variants of haplogroup N3 distinguished by a difference at DYS19 (Zerjal et al. 2001). Our study shows that high frequency of haplogroup N3 in Pskov population is caused by the presence of Baltic-specific STR-haplotypes (64 per cent of N3-chromosomes), whereas, in average, 80 per cent of N3 haplotypes found in the majority of Russian populations from central and southern regions of European Russia are characterized by Uralic-specific STRs. This work was supported by the Russian Fund for Basic Research (03-04-48162) and the Polish State Committee for Scientific Research (3P04C 04823, 2002-2005).

Haplotype diversity and sequence divergence in segmental duplications. *M.E. Hurles¹, S.J. Lindsay¹, S.E. Hunt¹, M. Khajavi², J. Lupski².* 1) Wellcome Trust Sanger Institute, Cambridge, United Kingdom; 2) Dept. of Molecular and Human Genetics, Baylor College of Medicine, Texas, USA.

The human genome appears enriched for segmental duplications compared to other mammalian and non-mammalian genomes. Many of these segmental duplications are sufficiently similar that they can be recognised as substrates for homologous recombination during meiosis. The resulting Non-Allelic Homologous Recombination (NAHR) events can generate disease-causing rearrangements and gene conversion mutations as well as structural polymorphisms. The complexity of studying high copy number sequences has caused the medical and evolutionary consequences of NAHR to be under-ascertained. This is especially true for studies of complex diseases and of the evolution of duplicated genes. Sequence variation among individuals is key to understanding both of these issues. It has been noted that single nucleotide polymorphisms (SNPs) in dbSNP are enriched within segmental duplications, although there is debate as to whether this apparent increase in sequence diversity is real or artefactual. We have developed a novel method for experimentally determining haplotypes within segmental duplications called Anchored Shotgun Haplotyping. We have applied this method to generating datasets of haplotypic variation in and around known hotspots of NAHR activity within several segmental duplications. We have characterised the sequence variation within humans as well as sequence divergence between humans and other hominoid species. These data are complemented with analyses of SNPs derived from flow-sorted chromosomes that map to inter-chromosomal segmental duplications. We find sequence variation to be significantly elevated within humans and between hominoids within specific portions of some segmental duplications. Simulations suggest that these data are best explained by localised gene conversion between segmental duplications. The complex haplotypic structures that are formed may confound association-based searches for genes underlying complex disease, but potentially facilitate the identification of rearrangement hotspots from comparative genome sequences.

The evolutionary conservation of four satellite III DNA subfamilies among primates. *M. Jarmuz*¹, *R.*

*Bandyopadhyay*², *C.D. Glotzbach*^{1,3}, *K.A. Bailey*^{1,3}, *A.L. Rosa*^{1,3}, *L.G. Shaffer*^{1,3}. 1) Health Research and Education Center, Washington State University, Spokane; 2) Department of Human and Molecular Genetics, Baylor College of Medicine, Houston, TX; 3) Sacred Heart Medical Center, Spokane, WA.

Satellite DNA likely plays an important role in eukaryote chromosome structure and karyotypic evolution through the facilitation of exchange events. In the present study, we analyzed satellite III DNA (satIII DNA) from two subfamilies characterized by their sequence composition: the GGAAT monomer sequence is predominant in the first group, whereas in the second a roughly equal representation of GGAAT and GGAGT motifs are found. Both groups are present in the short arms of the acrocentric chromosomes and in chromosomes 9 and Y in human and share high homology. In our study, we determined the distribution of four subfamilies of satIII DNA from group 1 (pTRS-63, pW-1) and two from group 2 (pR-4, pTRS-47) in 25 primate cell lines by FISH, Southern hybridization and PCR with primers specific to each subfamily. We also determined the presence or absence of the subfamilies in four human reference cell lines and a panel of monochromosomal hybrids of the acrocentric chromosomes. Using FISH with each subfamily as a probe we determined the chromosomal distribution and co-localization of satIII DNA with the rRNA genes in each species. These satIII subfamilies were not detected in squirrel monkey, african green monkey, baboon, or rhesus monkey but were found in orangutan, indicating that these subfamilies appeared suddenly in this species. The orangutan contains the largest number of satIII-bearing chromosomes (n~19), with a reduction in gorilla, chimpanzee and human to only 2-12 chromosomes, depending on the subfamily. These subfamilies co-localize with rDNA genes in all of the acrocentric chromosomes in orangutan, whereas in gorilla co-localization is present for only one chromosome pair or is absent depending on the subfamily. Chimpanzee and human share the most similar patterns with co-localization on one to five pairs of acrocentric chromosomes depending on the subfamily. These results show striking interspecies variability among primates and support the appearance of satIII DNA ~10 MYA in primates.

PTC-tasting and negative evidence for balancing selection. *K.K. Kidd¹, A.C. Davidson¹, A.J. Pakstis¹, W.C. Speed¹, L. Bartoshuk², V. Duffy^{2,3}, J.R. Kidd¹.* 1) Dept Genetics, Yale U. Sch. Medicine, New Haven, CT; 2) Dept Surgery, Yale U. Sch. of Medicine, New Haven, CT; 3) School of Allied Health Sciences, U. of Connecticut, Storrs, CT.

Recent studies identify the *TAS2R38* taste receptor gene on 7q as the primary influence on the classical PTC taste threshold polymorphism. One study concluded that human population frequencies support R.A.Fishers hypothesis that balancing selection maintains this polymorphism. We report a much larger and extensive global sampling (over 2000 individuals, 40 populations) than previous studies for 3 coding region SNPs and 3 flanking SNPs as well as sequence data for multiple individuals from each of several other species of apes. Five of 8 possible haplotypes for the coding SNPs occur in our samples with two very common haplotypes worldwide corresponding to the classical taster and non-taster phenotypes. A third common haplotype occurs in African populations (5%-44%) and two low frequency haplotypes are found in various African and European samples. The average frequency of the taster haplotype differs substantially across the populations of the major geographical regions about 0.45 in Africa, 0.5 in S.W.Asia, 0.4 in Europe, 0.6 in East Asia, and 0.8 in the Americas. High levels of LD characterize the coding region spanning ~1KB. Sequence results in 5 ape species indicate the presence of different, non-conserved polymorphisms for this gene. Non-taster, null alleles occur in chimpanzee and orangutan that differ from each other and from human non-taster alleles. While the pattern of 2 high frequency alleles outside of Africa is consistent with balancing selection, the frequencies vary significantly among populations. We conclude that weak purifying selection and random genetic drift may best explain the global distribution. Published studies have also implicated differences in taste perception with dietary choices that have important health outcomes. Our ability to characterize the *TAS2R38* receptor at the molecular level now make possible studies to disentangle the multiple factors affecting taste of bitter substances. Supported in part by NIH grants GM57672 and AA09379 (KKK) and NSF SBR-9632509 (JRK).

Evolutionary insights into drug metabolism: Evidence for positive selection in Drug Metabolism and Drug transporter genes. *H.A. Madsen¹, C.A. Sprankle², A.M. Saunders², N.K. Spurr², D.K. Burns², M.J. Stanhope¹, B.T. Koshy².* 1) Bioinformtic Sciences, Glaxo Smith Kline, Collegeville, PA-19426; 2) Genetics Research, Five Moore Drive, Research Triangle Park, NC-27709.

Most genes reported to be under diversifying selection involve host pathogen responses. There has also been evidence of diversifying selection on genes involved in olfaction, as well as on the tumor suppressor gene, BRCA1. We utilized a comparative evolutionary approach to understand genetic selection pressure involved in the phenotypic differences in drug metabolism between humans and species that are used for pharmacokinetic and toxicological evaluation of drugs such as rodents and non-human primates. We examined the sulfotransferases (SULT1A), a key enzyme family which plays an important role in the metabolism and bioactivation of many endogenous compounds and xenobiotics, as well as the ABCB1 drug transporter which has been implicated in the phamacokinetics of many drugs. The PAML (phylogenetic analysis using maximum likelihood) package was used to search for evidence of positive (diversifying) selection. The omega ratio (dN/dS ratio), a commonly used criterion for detecting adaptive evolution, quantifies the rate of non-synonymous to synonymous substitution; an omega>1 indicates that natural selection is favoring non-synonymous codon changes over synonymous codon changes. A comparison of SULT1A and ABCB1 mRNAs from ten mammalian species revealed several sites that had an ratio greater than 1. One of these sites, codon 89 in SULT1A, varies among human isozymes and affects substrate specificity. Our findings are intriguing and indicate that differential selective pressures operating on drug metabolizing enzymes and transporters may have important implications for drug discovery.

The CAG-repeat polymorphism of the DNA polymerase gamma gene in North Eurasian human populations. *B.A. Malyarchuk¹, M. Papuga², T. Grzybowski², M. Wozniak², M.V. Derenko¹, J. Czarny², I.A. Zakharov³, D. Miscicka-Sliwka².* 1) Institute of Biological Problems of the North, Magadan, Russia; 2) The Ludwik Rydygier Medical University in Bydgoszcz, Forensic Medicine Institute, Bydgoszcz, Poland; 3) Vavilov Institute of General Genetics, Moscow, Russia.

To estimate the level of variability of the nuclear gene for the catalytic subunit of mitochondrial DNA polymerase gamma (POLG), polymorphism of the CAG microsatellite repeat was studied in 1155 healthy individuals representing 18 populations of North Eurasia. These samples were grouped into three geographic areas: Europe, Southwest Asia and Siberia/East Asia. We have observed that 10-repeat allele of POLG gene is the most frequent in all populations studied, being found at frequency of 96, 93 and 88 per cent in Siberian/East Asian, Southwest Asian and European populations, respectively. The frequency of 11-repeat allele ranges from 9 per cent in Europe to 5.7 per cent in Southwest Asia and to 3.3 per cent in Siberia/East Asia. The remaining alleles ranging from 6 to 13 repeats were found at very low frequencies, with a maximum number of private alleles in Europe (6-8-repeats). The heterozygosity ranges from 22 per cent in Europe and 13.6 per cent in Southwest Asia to its lowest value of 7.4 per cent in Siberia/East Asia. Out of 13 observed POLG genotypes, only four of them are in common among human populations studied, but seven and two different genotypes were found as private ones, in European and Siberian/East Asian populations, respectively. Differences among regional groups account for 2 per cent of the total variation. Finally, the present study provides evidence for a clinal distribution of POLG gene heterozygosity in North Eurasian populations. This pattern could be related to the demographic history of human populations (including human migrations and genetic drift), meanwhile the effect of balancing selection (at least, in European populations) should also account for the observed differences. This work was supported by the Ludwik Rydygier Medical University in Bydgoszcz (grant BW 61/04) and by the grant from the Far-East Branch of the Russian Academy of Sciences (04-3-A-06-039).

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Treescan: A method using haplotype networks to direct genotype/phenotype association studies. *T.J. Maxwell, A.R. Templeton, MICORTEx*. Department of Biology, Washington University in STL, St. Louis, MO.

Tree-scanning walks across a haplotype network using each side of an evolutionary branch to define a two-allele system. The first step is to build a phylogenetic network and test for recombination. The resulting nonrecombinant network is used to define a set of *a priori* contrasts by splitting each branch to divide the haplotypes into two groups of alleles. Phenotypic permutations across the resulting genotypes defined by the two alleles are used to find the significance of each contrast (different statistics for discrete or continuous data) with a multiple tests correction that retains the correlation structure. If a significant contrast is found, a conditional permutation procedure is used to cut a second branch while keeping the first branch cut to split the haplotypes into a three allele system. Tree-scanning preserves statistical power by pooling haplotypes while minimizing the number of tests with an evolutionarily relevant *a priori* criterion. It provides an interpretive framework that can recognize and correct for homoplasy, identify multiple independent effects within a region, potentially localize physical regions with rare recombinants, and can be extended to multilocus epistasis studies. Examples are demonstrated with a large data set of human cholesterol related phenotypes and ApoE genotypes.

Detecting signatures of natural selection in skin pigmentation candidate genes using locus specific pairwise F_{ST} .

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Pigmentation of the skin is an easily visible example of human phenotypic variation. Natural selection may have shaped global variation in human skin pigmentation by acting on genes controlling the production and distribution of melanin, the primary pigment of the skin. Melanin acts as a natural sunscreen, suggesting that large amounts of melanin in the skin may be adaptive in environments where ultraviolet radiation is high and that lower levels of melanin may be adaptive in the higher latitudes where ultraviolet radiation is not as strong. Recent studies suggest that SNPs in some pigmentation candidate genes may have an effect on normal variation in human pigmentation. This work examines locus specific pairwise F_{ST} values ($lspF_{ST}$) for SNPs in 5-6 pigmentation candidate genes in pairwise comparisons of six geographically diverse human populations. These values are then compared to an empirical $lspF_{ST}$ distribution based on 11,078 autosomal SNPs typed in the same or similar population samples. High $lspF_{ST}$ values may be suggestive of population differentiation due to the effects of natural selection, although these high values may also have been caused by genetic drift. Pigmentation candidate SNPs with high $lspF_{ST}$ values between populations known to differ in skin pigmentation suggest that the SNP may have a functional effect on pigmentation. High $lspF_{ST}$ values between populations with similar pigmentation phenotypes may suggest convergent evolution. Two such SNPs showing high $lspF_{ST}$ values between European and East Asian populations were identified in a preliminary population screen. Where possible, levels of LD surrounding putative selected SNPs will be examined to help determine if high $lspF_{ST}$ levels are due to drift or to natural selection.

Patterns of nucleotide sequence diversity among humans and great apes at the Arginine Vasopressin Type II Receptor Gene (*AVPR2*). *L.A. Pfeifer*¹, *B.C. Verrelli*², *S.A. Tishkoff*¹. 1) Department of Biology, University of Maryland, College Park, MD; 2) Center for Evolutionary Functional Genomics, School of Life Sciences, Arizona State University, Tempe, AZ.

The primary action of the peptide hormone arginine vasopressin (AVP) is antidiuresis. Binding of AVP to the type II AVP receptor (*AVPR2*) in the kidney allows for reabsorption of body water and electrolytes. Mutations in the *AVPR2* gene encoding this G-protein coupled receptor, located at Xq28, cause the disease X-linked nephrogenic diabetes insipidus (NDI). While a number of studies have identified *AVPR2* mutations in individuals with NDI, nucleotide sequence variation at this gene has not been previously investigated in a random sample of individuals from ethnically diverse populations. Here, we present the analysis of 2,500 bp of *AVPR2* and its proximal promoter region from over 150 humans originating from indigenous populations of: Eastern, Western, and Southern Africa, the Middle East, Europe, Asia, Australo-Melanesia and the Americas. We also assessed *AVPR2* in a sampling of great ape species. Thus far we have identified 19 single nucleotide polymorphisms (SNPs) in humans, including nine novel SNPs not previously reported. Five of these SNPs result in amino acid (aa) replacements, which may have functional implications. Notably, two of the aa changing SNPs found in this study were previously reported as disease-causing mutations in individuals with NDI. We also observe 68 fixed differences between the human and great ape lineages; nine of these are aa fixed differences. Because *AVPR2* plays a critical role in osmoregulation, we have tested for evidence of selection acting at this locus across human populations originating from ecologically and geographically diverse regions. This study provides estimates of *AVPR2* nucleotide and haplotype diversity that may be useful for disease association studies, for reconstructing the history of *AVPR2* protein evolution since the split of humans from their most recent common ancestors, and for studying the genetic basis of adaptation in humans.

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Evidence of Balancing Selection at the Promoter Region of *HLA-G*. Z. Tan, K. Vigh, C. Ober. Department of Human Genetics, The University of Chicago, Chicago, IL 60637.

The primary site of expression of *HLA-G* is at the fetal-maternal interface. Because of its immunosuppressive properties and limited polymorphism in the coding region, *HLA-G* is thought to contribute to maternal tolerance of the fetus. Recently we reported 18 SNPs in the 1350 bp upstream of exon 1, which includes all the known regulatory elements, an unusual haplotype structure, and an association with miscarriage in the Hutterites (Ober C, et al. *AJHG* 72:1425). To further study this region, we used haplotype-specific PCR and sequenced the promoter in 44 African Americans (AA), 44 Caucasians (CA) and an orang. We identified 24 SNPs, comprising 10 unique haplotypes (9 in AAs; 8 in CAs) that fell into 2 divergent clades. Tajimas D was 2.56 in the AAs ($P=0.005$) and 2.20 in the CAs ($P=0.02$); nucleotide diversity was high ($=0.006$ in both). These features are consistent with the presence of an old balanced polymorphism. To further test this hypothesis, we sequenced in the orang and AAs 1.3 kb downstream of the promoter region, between which there was little LD. We performed an HKA test, which revealed greater than expected diversity in the human promoter region ($P<0.05$). These combined data are consistent with the hypothesis that the *HLA-G* promoter region has been subject to balancing selection, perhaps due to the opposing needs to protect the fetus from rejection and maintain immune competence at the maternal-fetal interface. Supported by HD21244 and HL72414.

Functional analysis of protein-altering variants of membrane transporters in human populations. *T.J. Urban¹, M.K. Leabman¹, R. Sebro², N. Risch², K.M. Giacomini¹*. 1) Biopharmaceutical Sciences, UCSF, San Francisco, CA; 2) Department of Genetics, Stanford University School of Medicine, Stanford, CA.

Although considerable progress has been made toward characterizing the nature and degree of human DNA sequence variation, there remains a general deficiency in information on human phenotypic variation at the single-gene level. We systematically analyzed the function of all protein altering variants of nine membrane transporters in heterologous expression systems. Coding region variants were identified by screening a large sample (n = 247 to 276) of DNA from ethnically diverse subjects. In total, we functionally analyzed 79 protein altering variants, including 75 non-synonymous variants, two insertions, one deletion, and one nonsense mutation. Ten percent of all tested protein-altering polymorphisms (i.e., variants found at allele frequencies greater than 1% in at least one major ethnic group) were non-functional or had significantly reduced function, defined as <50% of activity compared with the reference sequence. Of the 79 variants studied, 16 (18%) had significantly decreased function. Decreased function variants had a significantly different allele frequency distribution (i.e., a skew toward lower population allele frequencies for decreased function variants) (p=0.01, log rank test) and were more likely to alter evolutionarily conserved amino acid residues (p=0.00913, logistic regression) than variants that retained function. Among variants that retained function, evolutionary conservation was also a significant predictor of the degree of change in function (p=0.04955, log rank test). Collectively, these data indicate that variants that have grossly impaired function are present at lower allele frequencies than those that do not, and that function in cellular assays is the best predictor of human fitness. The data suggest that variants that alter evolutionarily conserved residues but retain function in cellular assays may yet influence occult functions or quantitative degrees of function that are important in human fitness.

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Signature of natural selection revealed at HLA region with microsatellites: Further Data. *H. Xu*¹, *R. Deka*², *M. Kimmel*³, *Y.X. Fu*⁴, *R. Chakraborty*². 1) Dept Epidemiology, Univ Texas MD Anderson CA Ctr, Houston, TX; 2) Center for Genome Information, Department of Environmental Health, University of Cincinnati, Cincinnati, OH; 3) Department of Statistics, Rice University, Houston, TX; 4) Human Genetics Center, School of Public Health, University of Texas, Houston, TX.

Natural selection is one of the major factors in the evolution of all organisms. Detecting the signature of natural selection has been the central theme in evolutionary genetics. With the availability of microsatellite data, it is of interest to study how natural selection can be detected with microsatellites. Currently used tests of natural selection presume that mutations at the loci follow the infinite allele/site models, in the sense that at each site at most only one mutation event is recorded, and each mutation leads to an allele not seen before in the population. Microsatellite loci do not obey these mutation models, since the new alleles at such loci can be created either by contraction or expansion of tandem repeat sizes of core motifs. We developed a new test of neutrality taking account of a generalized model of forward-backward stepwise mutations. As confounding factors of natural selection, the effects population expansion and population substructure on the test were also studied. Applying the new test to the microsatellite loci from human HLA region revealed the signature of natural selection.

Mutation rate and variation patterns among 16 SARS coronavirus genomic sequences. Z. Zhao^{1,2}, Y.-X. Fu³. 1) Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University, Richmond, VA; 2) Center for the Study of Biological Complexity, Virginia Commonwealth University, Richmond, VA; 3) Human Genetics Center, University of Texas Health Science Center at Houston, Houston, TX.

The outbreak of severe acute respiratory syndrome (SARS) caused a severe global epidemic in 2003 and linked to hundreds death and thousands in hospitals. The virus causing SARS was identified as a novel coronavirus (SARS-CoV) and multiple genomic sequences were revealed since mid-April, 2003. Understanding the evolutionary mechanism of SARS-CoV, including mutation rate and pattern of variations, is fundamental for battling against the deadly pathogen. We analyzed 16 complete genomic sequences with available clinical histories during the SARS outbreak. After careful examination of multiple-sequence alignment, 114 single nucleotide variations were identified. The properties of amino acid changes in the different genomic regions were analyzed and compared. The reconstructed phylogeny indicated two subgroups, one isolated in mainland China and the other included those connecting to index patient A. We applied three strategies to infer the mutation rate in SARS-CoV genome by using the 11 genomic sequences for which the divergent time can be inferred. The mutation rate was estimated to be $0.80 - 2.38 \times 10^{-3}$ nucleotide substitution per site per year which is in the same order of magnitude of other RNA viruses. The estimates of mutation rates led to the inference that the SARS-CoV could have been with humans in the spring of 2002 without causing a severe epidemic. The synonymous and nonsynonymous substitution rates were estimated to be $1.67 - 4.67 \times 10^{-3}$ and $1.16 - 3.30 \times 10^{-3}$ per site per year, respectively. The genome-wide Ka/Ks ratio indicated little selective pressure, but a few genes might have been subjected to very recent natural selection.

Maternal Thrombotic Risk Factors For Small Gestational Age. *F.B. Atac¹, N. Ozbek², H. Verdi¹, S. Cetintas², E. Ozyurek², F.I. Sahin¹, B. Gurakan², A. Haberal³.* 1) Medical Biology and Genetics, Baskent University Faculty Of Medicine , Ankara-Turkey; 2) Pediatrics, Baskent University Faculty Of Medicine, Ankara-Turkey; 3) Obstetrics and Gynecology, Baskent University Faculty Of Medicine, Anaka-Turkey.

Altered maternal homeostasis as a result of point mutation or single nucleotide polymorphism in coagulation factors has been reported as a new and frequent risk factor in Small for Gestational Age (SGA), in addition to several fetal and placental factors. Therefore, we assessed whether maternal thrombophilic risk factors such as, Factor V Leiden (FVL), Prothrombin G20210A (Prt G20210A) and methylene tetrahydrofolate reductase (MTHFR) C677T mutations are associated with SGA in Turkish population. 57 maternal blood samples from mothers having babies with SGA formed the study group of this study. Control group was consisted of 67 maternal blood samples withdrawn from women without any obstetric complication. Every patient included in this study was informed about the DNA analysis consequence of the withdrawn blood sample and was requested to sign the written informed consent form. Genomic DNA was prepared from leukocyte pellets by sodium dodecyl sulphate (SDS) lysis, ammonium acetate extraction and ethanol precipitation. Restriction fragment size analysis was performed by visualising digested PCR products for FVL, Prt G20210A, MTHFR C677T mutations, and separated by electrophoresis in an ethidium bromide stained 12% polyacrylamide gel. Based on the preliminary findings obtained from the present work (Table 1), we concluded that MTHFR C677T mutation may be suggested as a risk factor for the SGA outcome in our population and screening could help the assesment of patients.

Gene mutation	Study Group (n=57)	Control Group (n=67)
FVL (WT)	100% (n=57)	98.50% (n=66)
FVL (Heterozygote)	-	1.50% (n=1)
Prt G20210A (WT)	100% (n=57)	97.00% (n=65)
Prt G20210A (Heterozygote)	-	3.00% (n=2)
MTHFR C677T (WT)	57.89% (n=33)	68.65% (n=46)
MTHFR C677T (Heterozygote)	40.35% (n=23)	29.85% (n=20)
MTHFR C677T (Homozygote mutant)	1.75% (n=1)	1.50 (n=1)

Genetic diversity in the coding regions of three members of the glutathione peroxidase family. *C. Leger¹, M. Simoneau¹, M.-C. Theberge¹, H. Vasquez¹, J.-F. Lefebvre¹, E. Levy^{1,3}, D. Sinnott^{1, 2}, D. Labuda^{1,2}.* 1) Hopital Sainte-Justine, Universite de Montreal, Montreal, Quebec, Canada; 2) Departement de Pediatrie, Hopital Sainte-Justine, Universite de Montreal, Montreal, Quebec, Canada; 3) Departement de Nutrition, Hopital Sainte-Justine, Universite de Montreal, Montreal, Quebec, Canada.

Reactive oxygen species (ROS), while having recognized functions, may also cause damage to the cell. Three members of the glutathione peroxidases family, GPX1 (or cGPX), GPX2 (GPX-GI) and GPX3 (plasma GPX), appear at the first line of defense against ROS. Thus, polymorphisms in the corresponding genes, may represent genetic susceptibility factors. In this study, we characterized variations within the coding sequence of GPX1, 2 and 3 in a worldwide panel of 180 DNA samples by dHPLC analysis, sequencing and subsequent genotyping of variants. These genes differ substantially in their variability in human populations. Five SNPs (nucleotide diversity $h=0.07\%$) and a tri-nucleotide repeat (oligoalanine tract) were found in GPX1; 18 SNPs ($h=0.12\%$) in GPX3; and only 3 singleton SNPs ($h=0.002\%$) in GPX2. All substitutions appeared neutral, with the exception of a P to L substitution located at a phylogenetically variable C-end terminus GPX1. Among 7 GPX1 haplotypes, further diversified by tri-nucleotide repeat, three accounted for 98% of all 294 chromosomes tested; in GPX3 five haplotypes out of 19 observed represented 85% of the sample. Virtual absence of diversity in GPX2 was neither correlated with its evolutionary conservation, since its divergence from the chimpanzee ($\sim 1\%$) was comparable to that of GPX3, nor with a lower regional recombination rate (1.6 cM/Mb). Interestingly, in GPX3 Asians were statistically different from other population group essentially due to partition of major haplotypes, H15 in Asia and H12 elsewhere. Of note, there is a positive correlation between the rate of hepatocarcinoma and the population frequency of GPX3 haplotypes harboring opposite SNP1071 alleles (i.e. H12 and H15) suggesting its possible association with this disease. Supported by Valorisation Recherche Quebec.

CYP3A4 gene polymorphism in renal transplant recipients and the association with cyclosporine clearance. *D.O. McDaniel, S. Harrison, W.H. Barber, L.S. McDaniel.* Dept Surgery/Neurology, Univ Mississippi Medical Ctr, Jackson, MS.

CYP3A4 plays an important role in the metabolism of immunosuppressive drugs, including cyclosporine. Most organ transplant recipients in addition to immunosuppressive drugs also take medications for other conditions. The administration of multiple drugs might have an adverse effect in drug distribution following long-term drug treatment. Because of variability in the drug uptake and clearance, this might have a great impact on the outcome of allograft survival. We have demonstrated interindividual variation in CYP3A4 expression in different individuals. Such variation is hypothesized to be due to polymorphism within the promoter region of the gene causing difference in the level of expression. The aim was to determine the allelic frequency of the CYP3A4 variants in African-American kidney recipients, and to examine a possible association with cyclosporine elimination in the transplant setting. Blood samples from 77 patients and 67 matched controls were studied by single nucleotide polymorphism (SNP) and PCR. CYP3A4 G genotype was present in 82.5% of renal transplant patient population. The GG homozygous allele was 4-fold higher in patients than in controls. The frequency of G was significantly higher in the Mississippi population (82.5%) as compared with African-American population (53%) reported elsewhere. There was a trend towards higher cyclosporine clearance index and AA genotype, indicating that the effect of CYP3A4 might be more evident in a homozygous GG genotype. Such information might allow improved strategies for immunosuppressive drugs such as cyclosporine and tacrolimus that are commonly used to prevent allograft rejection.

Fine linkage disequilibrium mapping using the coalescent with recombination. *F. Larribe*¹, *K. Morgan*². 1) Human Genetics, McGill University, Montreal, Quebec, Canada; 2) Human Genetics and Medicine, McGill University, Montreal, Quebec, Canada.

We present new developments on a multilocus fine mapping linkage disequilibrium methodology using the ancestral recombination graph (ARG) to model the history of related haplotypes. A trait influencing mutation (TIM) is assumed to have occurred in one haplotype of the population some time in the past. This mutation is assumed to be unique (non recurrent) and to have a high penetrance. Based on a sample of cases and controls from this population, the method evaluates the likelihood of the position of the TIM along the sequence, leading to a maximum likelihood estimator. The history of the population, based on both cases and controls, is modelled by the coalescent process with recombination, which is probably a better model than other previously used models like star genealogies or trees. The evaluation of the likelihood is done with the importance sampling method of Griffiths and Tavar which we have adapted to this context. The method can be used with SNPs or micro-satellite markers and can take into account variable population size. This computationally intensive Monte Carlo method should be generalizable to others contexts, as it is directly based on the coalescent process. We present results of simulations and results on a real data set. It is well known that inference in the ARG is difficult, and we show that despite some variability in the likelihood profiles, the method works well and is promising. We discuss some problems, and finally present some future research directions.

Genetic Diversity in Two Urban Populations on the Southern Coast of Ghana. *J. Benn Torres*¹, *A.C. Stone*². 1) Dept Anthropology, Univ New Mexico, Albuquerque, NM; 2) Dept Anthropology, Arizona State Univ, Tempe, AZ.

Understanding genetic diversity in West Africa is of importance because it provides insight into African population history and overall human diversity, as well as into the history of the African Diaspora. Studies regarding genetic diversity in West African populations are comprehensive but generally reference continental population history, disease, or species origin and do not usually consider urban diversity. In this analysis mtDNA hypervariable region I (HVI) and four Y chromosome markers are examined in 152 unrelated individuals representing nine different ethnicities from two metropolitan areas, Cape Coast and Accra, in Ghana, West Africa. This investigation highlights genetic diversity and examines the relationship between tribal affiliation and genetic markers. In addition, comparisons concerning diversity relationships are made between these data and those published from other African and African- derived populations. The DNA samples in this study were collected via cheek cell swabs, extracted using a standard phenol-choloroform method, and amplified by PCR. Electrophoresis, sequencing, or Taqman, were used to determine the genotype for each locus. The heterozygosity and frequencies for each of these markers were calculated and compared with previously published data. Preliminary analysis at several of the loci indicated, firstly, that more analysis is need to ascertain the relationship between genetic haplotypes and tribal affiliation, and second, that overall, the genetic diversity observed in these urban samples are higher than in other non-urban West African populations.

Study of Phylogenetic relations of two North Indian Populations based on Mitochondrial & Y Chromosomal DNA Polymorphisms. *A.J.S. Bhanwer¹, K. Thangaraj², S. Sharma³, R. Singh¹, L. Singh².* 1) Department of Human Genetics, GND University, Amritsar; 2) Centre for Cellular & Molecular Biology, Hyderabad; 3) National Centre for Applied Human Genetics, JNU, New Delhi.

The human nature to explore has led us for the quest of unravelling the mystery of our origins. The Out of Africa theory of human origin and migration and its supporting concepts suggest India as one of the most interesting global regions in human evolution. Some of the most ancient waves of human migration appear to have passed through India & North India is considered as the Melting pot of the Indian populations & has an important role in peopling of India. Due to their property of uni-parental inheritance mtDNA and Ychromosomal markers are most effective molecular genetic tools for these evolutionary and phylogenetic studies. The current study traces phylogenetic relationship of two North Indian populations Kashmiri Pandits & Ramgarhia Sikhs with other Indian and world populations using these markers. The Y-biallelic markers based haplogrouping for both these populations show a great genetic affinity as the major haplogroups found are F(0.230 & 0.36),O(0.11 & 0.18),K(0.11 & 0.18),R(0.29 & 0.28) and Q(0.26 & 0.0) in Kashmiri Pandits and Ramgarhia Sikhs respectively and show almost equal haplogroup distribution pattern. The absence of haplogroup Q in Ramgarhia Sikh population indicates an ancient population admixture and recent population specification which may occur due to recent population and caste specific socio-culture differentiation. 9bp polymorphism is absent. Both show close affinity to Vanuatu (Melanesian), a rare Polynesian population by neighbour joining method based on F_{st} distance values of mtDNA HVR I data from two population groups and other world population. The mismatch pair distribution analysis based on HVR I sequence data show a population expansion in both the population groups but a more ancient expansion in Kashmiri Pandits than Ramgarhia Sikh population. Analysis of 4 mtDNA bi-allelic markers in Ramgarhia Sikhs showed the presence of mtDNA haplogroups M and R in high frequency along with haplogroup U to some extent indicating a conserved maternal inheritance.

Presence of deep-rooted YAP lineages in some population groups of Jammu and Kashmir. *A.K. Bhat^{1,2}, S. Sharma¹, A. Saha¹, M. Dhar², R. Bamezai¹.* 1) NCAHG, SLS, Jawaharlal Nehru University, New Delhi, Delhi, India. ; 2) Department of Biotechnology, University of Jammu, J&K, India.

There is a complete non-availability of the genetic analysis to reflect upon genetic relatedness of different population groups of the state of Jammu and Kashmir in India. Ancient documentations such as Nilmatpuran and Ragtarangni do throw some light on early peopling of the region but it is inconsistent with excavation done at Burzihama in Kashmir. Since, Y Chromosome and mitochondrial genome analysis have proved of help in tracing the origin of a population, this study was carried out on 168 samples [133 male, 35 female] from three major population groups, representing Kashmiri Pandits [KPs], Kashmiri Muslims [KMs] and Ladakhis. KPs are believed to be the true representatives of Aryans whereas, the majority of KMs are believed to be the descendants of converted Hindus and the Ladakhis are considered close to Tibetians. The Y chromosomal markers: 12 biallelic and 4 Y-STRs and the two mitochondrial variable regions, HVRI and HVRII, were analysed. A deep-rooted African YAP+ lineage at a frequency of 2.5% in KMs, hinted towards some sort of antiquity in the peopling of Kashmir. The Tibetan lineage of YAP+ chromosomes [9.5%] was found in the Ladakhi population. The other clades (YCC) were: clade C, 3.8% in KPs, 2.6% in KMs; clade J, 4% in KPs, 13% in KMs and 4.6% in Ladakhis and clade R1a, 15.3% in KPs, 18% in KMs. The Y-STR data reflected that the studied populations are close to rest of the Indian populations. A molecular variance of 72.68% and 67.02% was observed at HVRII and HVRI loci respectively among population within groups. The presence of almost similar clades in both KPs and KMs suggested a common ancestry for both populations. The presence of Tibetan lineage in Ladakhi population probably reflected the geographical and cultural proximity of the two populations. Whether this lineage migrated from Ladakh to Tibet or vice-versa needs to be explored. The presence of an African YAP+ lineage though in less frequency was a unique finding which in future studies will be helpful in tracing the human migration from Africa into Asia through Kashmir.

Polymorphisms in Genes of Glutathione Metabolism: Promoter Regions. C. Labbe¹, C. Moreau¹, V. Yotova¹, D. Gehl¹, J.F. Lefebvre¹, P. Beaulieu¹, S. Langlois¹, M.C. Théberge¹, H. Bélanger¹, C. Zotti², D. Sinnott¹, D. Labuda¹, *Regulatory Genetics Consortium*. 1) Hemato-Oncology Center, Sainte-Justine Hospital, Montreal, QC, Canada; 2) McGill University and Genome Quebec Innovation Centre, Montreal QC Canada.

Regulatory polymorphisms that modify genetic expression may have an impact on the susceptibility to diseases and/or on the differential response to treatment. Enzymes of glutathione metabolism are of special interest because of their involvement in detoxification of the cell and its protection against reactive oxygen species. To learn about genetic diversity in their bona fide promoter regions of the corresponding genes, we characterised variability of 2 kb segments upstream of the transcription start site. Polymorphisms were ascertained in a world sample of 40 individuals and studied further in an extended sample of five continental populations (n = 80) and 8 subpopulations (n=142). Three genes have been studied so far: nucleotide diversity of 0.08% was found in GSTT1 and GSS (with 6 and 9 segregating sites, and 6 and 11 haplotypes, respectively), and of 0.21% for GSTP1 (13 sites and 16 haplotypes). The most frequent haplotype of GSTT1 (44%) is a deletion of the entire gene. The remaining haplotypes diversity at 0.55 is lower than in GSS (0.71) or GSTP1 (0.71). While GSTT1 and GSS haplotypes can be explained entirely through mutations, the GSTP1 haplotype network indicates recombination episodes. PC analyses based on haplotype frequency in continental populations demonstrate striking differences between Africa and the rest of the world for each one of these genes. Although no deviation from neutrality was detected by Tajimas and Fus tests in neither of the segments analysed, an elevated FST of 0.288 in the case of GSTT1 suggests that selection had an impact on the evolution of this gene. The work on other GS-transferases (GSTM1, GSTM3, GSTM4) is in progress. Supported by Genome Quebec/Canada.

Population genetic of *bona fides* regulatory regions. D. Labuda¹, C. Moreau¹, S. Langlois¹, D. Gehl¹, P. Beaulieu¹, J.F. Lefebvre¹, H. Vasquez¹, C. Labbé¹, M.C. Theberge¹, H. Bélanger¹, C. Zotti², D. Sinnott¹, Genome Quebec Regulatory Genetics Consortium. 1) Res Ctr, St-Justine Hosp, Univ Montreal, Montreal, PQ, Canada; 2) Genome Quebec Innovation Ctr, McGill University, Montreal, PQ, Canada.

Diversity of human DNA is currently under intense scrutiny, leading us to better understanding of human origins, evolution, demographic history and population structure, essential to genetic epidemiological quest of complex diseases. In fifteen candidate genes, the promoter regions, arbitrarily defined as 2 kb segments directly upstream of the transcription start site, were screened by dHPLC in 40 individuals of African, Middle-Eastern, European, East Asiatic and Amerindian descent. The polymorphisms were characterized by sequencing and subsequently genotyped in an extended panel of 80 individuals representing the same population groups. We found between 3 and 18 (average of 10) segregating sites in the 2 kb regulatory region. Nucleotide diversity estimates of 0.03 to 0.19 %, from both allele frequencies (average 0.08%) and the number of segregating sites (0.1%) fit well the genomic average. Haplotype worldwide diversity was 0.65 (0.27 to 0.84) and their numbers from 4 to 21 (average 10) correlated with the count of segregating sites, as if recombination played little role in diversifying these haplotypes. Yet, in one third of these segments, recombinations (gene conversions) could have been documented suggesting that some of these putative regulatory segments recombine more often than the genomic average and could thus reside in the recombination hot spots. The partitioning of haplotype diversity varies substantially among continental groups suggesting the possibility of diversifying and/or balancing selection acting on these genomic segments. Besides advancing our knowledge of the promoter regions, knowing these characteristics will find application in planning the association studies and in using the candidate gene approach in particular. Supported by Genome Quebec/Canada and by Valorisation Research Quebec.

Haplogroup U5a: the signature of an Upper Palaeolithic migration from West to East Eurasia? *K.P. Mooder¹, T.G. Schurr², F.J. Bamforth¹*. 1) Human Identification Laboratory for Archaeology, Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada; 2) Department of Anthropology, University of Pennsylvania, Philadelphia, PA.

The prehistory of Siberia is still relatively uncharacterised, including the population origins of those who initially inhabited the region. One intriguing facet of Siberian prehistory was the Upper Palaeolithic occupation of the Lake Baikal region at sites known as Mal'ta and Buret'. Archaeological excavations revealed these groups to share a remarkably similar material culture with those living contemporaneously in eastern Europe; however, insufficient numbers of human remains were discovered at these sites to make inferences about their population affinities. We provide potential support for a West Eurasian contribution to the gene pool of prehistoric Siberia with the mtDNA analysis of two Neolithic Lake Baikal cemetery populations. Dating from 6125 to 2020 B.C., these two groups not only possess a wide variety of East Eurasian mtDNA haplogroups but also harbour HVI polymorphisms characteristic of the West-Eurasian haplogroup U5a at frequencies of 5 to 10%. A survey of prehistoric mtDNA datasets reinforces the presence of U5a in the matrilineal structure of East-Eurasia and North America prior to European contact. Given these observed archaeological and biological affinities, it will be worthy to explore whether the dispersal of U5a and other West Eurasian haplogroups in prehistoric and modern East Eurasian populations provides insight into how the matrilineal population structure of this region has evolved.

Mitochondrial DNA polymorphisms in four ethnic groups from Mongolia. *B. Munkhbat*^{1, 2}, *H. Hayashi*¹, *K. Tounai*¹, *T. Katoh*¹, *G. Oyungere*², *N. Munkhtuvshin*², *H. Inoko*¹. 1) Dept Molecular Life Science, Tokai University School of Medicine, Isehara, Kanagawa, Japan; 2) Central Scientific Research Laboratory, National Institute of Medicine, Ulaanbaatar, Mongolia.

An analysis of mitochondrial DNA (mtDNA) polymorphism has been an essential tool for human diversity and molecular evolution studies allowing the insights of maternal lineage contributions. Here we will demonstrate the results of our comprehensive mitochondrial DNA analysis in Mongolians with the purpose to reveal matrilineal genetic affinity among contemporary Mongolians. The extended population samples including 475 unrelated individuals representing four indigenous populations, Khalkh, Uriankhai, Zakhchin and Khoton-Mongolians, are analyzed in present study. In brief, more than 50 haplotype-associated polymorphic sites comprising sequence variations in HVS-I, HVS-II and the coding region are investigated by direct sequencing, RFLP, and newly designed TaqMan probe assays in order to identify composite genetic structure of Mongolian ethnic groups from the view of mtDNA haplogroups. Moreover, highly complex genetic characteristics of four Mongolian ethnic groups will be discussed on the basis of extensive analysis of combined data from Y-chromosomal, X-chromosomal and mtDNA diversity studies in overall. In addition, the comparative analysis with the data available from neighboring populations from Central and Eastern Asia would be considered.

Frequency of mtDNA Haplogroups in five Mexican indigenous populations. *R. Peñaloza¹, IB. Alvarez¹, L. Buentello², I. Mendoza³, G. Gonzalez-Valencia³, J. Torres³, F. Salamanca¹.* 1) Unit of Human Genetics, IMSS, Mexico City; 2) IIA, UNAM; 3) Unit of Infectious Diseases, IMSS, Mexico City.

Abstract: Mitochondrial DNA (mtDNA) haplogroups from five indigenous Mexican groups not related, were analysed previous consent informed. We extracted DNA from leukocytes of peripheral blood by conventional methods and amplified five regions of mtDNA by PCR and finally used restriction enzyme as described previously (Moraga et al., 2000). We found the four American haplogroups with different frequencies. The results show that Nahuas from Milpa Alta presented the highest frequency of D haplogroup, probably because a special race mixture or high endogamy. The haplogroup A was frequent in Nahuas from Guerrero, Otomis from Hidalgo, Purepechas from Michoacan and Tzeltales from Chiapas. These results are in agreement with other authors.

The 9-bp deletion in the region V of mitochondrial DNA in several population of Zulia State-Venezuela. *L. Pineda, M. Portillo, L. Borjas, M. Sanchez, W. Zabala, E. Fernandez.* Medical Genetic Unit, University of Zulia, Maracaibo, Venezuela.

Zulia state, at the western of Venezuela, is considered a product of european and african admixture with the local amerindians, however, these are marked differences in this mixture according to the population studied. In this research, as a part of a great proyect to study genetic diversity of Zulia State, we report the preliminary results about the amerindian femenine contribution in several population of this state. Therefore, it was analyzed by PCR, the presence of deletion of one of the two copies of 9 bp in the V region of the mitochondrial DNA, that defines the amerindian haplogroup B in a sample of 266 individual coming from three populations of Zulia: Maracaibo, capital of the state, San Jose de Heras, a negroid group and Wayuu, representing the Arawak amerindian linguistic group. The deletion was present in all of the populations: 15%, 5% and 47%, respectively. The low frequency of this haplogroup in Maracaibo and San Jose de Heras suggests the presence of others amerindian haplogroups or a low amerindian contribution with an important contribution of others ethnic groups in these populations. The frequency observed in Wayuu group may be reflecting the presence of haplogroups derived of intermarriage processes with others local amerindians and mixed groups with different mitochondrial DNA. These results are agreed with the historical and demographic reports about the Zulia and demostrated that the deletion of 9 bp was useful to track through the time, the amerindian femenine contribution to the contemporary populations of the Zulia State.

Haplotype Diversity of the Y-chromosome in Six Mexican Populations. *H. Rangel-Villalobos*¹, *L.A. Riberos*¹, *L. Sandoval*², *L.E. Figuera*². 1) Molecular Genetics Laboratory, Universidad de Guadalajara, Ocotlan, Jal., Mexico; 2) Centro de Investigacion Biomedica de Occidente (CIBO-IMSS), Guadalajara, Jal., Mexico.

The non-pseudoautosomal region of the Y-chromosome constitutes a genetic record easily interpretable to obtain valuable anthropological information about the history of worldwide populations. Two bi-allelic loci (YAP and DYS199) and five STRs (DYS19, 389a, 390, 391 and 393) of the non-pseudoautosomal region of the Y-chromosome were analyzed in males from the largest and most widely distributed population in Mexico (Mestizos) and from three Mexican Amerindian tribes: Huichols, Purepechas and Tarahumaras. The allelic distribution of all seven loci was established and it was pairwise compared between populations. For YAP locus, any significant difference ($p > 0.05$) was observed among all four populations. The Amerindian-specific allele DYS199-T was more frequent in Mexican tribes than in Mestizos, establishing the minimum Amerindian component in the Mestizo sample as 18.6%. Tarahumaras were peculiar by its diminished frequency for DYS199-T respecting to Purepechas and Huichols. Mexican Mestizos were different ($p < 0.05$) to Huichols, Purepechas and Tarahumaras in five, four and two STRs, respectively. Eighty-eight different haplotypes were observed among the 156 haplotypes obtained. They were grouped in three haplogroups according to the markers YAP (+/-) and DYS199 (C/T):-/C, -/T and +/C. The greater haplotype diversity (D) was observed in Mestizos (98.6 %) and the lower in Huichols (87.17 %). The haplotype variation of the Y-chromosome in Mexican populations was analyzed by AMOVA. The inter-population and intra-population variations were significant ($p < 0.0001$) and constituted the 78.5% and 21.5%, respectively. We discuss our findings with previous results about the same populations using autosomal markers (Hum. Biol. 2000, 72: 983-995).

Serial segmental duplications during primate evolution result in complex human genome architecture. P.

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The human genome is particularly rich in low-copy repeats (LCRs) or segmental duplications (5-10%), and this characteristic distinguishes us from lower mammals such as rodents. How and why the complex human genome architecture consisting of multiple LCRs has evolved, remains an open question. Using molecular and computational analyses of human and progenitor primate genomic regions, we analyzed the structure and evolution of LCRs that resulted in complex architectural features of the human genome in proximal 17p. We found that multiple LCRs of different origins are situated adjacent to one another, whereas each LCR evolved at different time points > 25 to 3-7 million years ago, during primate speciation. Evolutionary studies in primates indicated abundant communication between the LCRs by gene conversion. The DNA transposable element MER1-Charlie3 and retroviral ERVL elements were identified at the breakpoint of the t(4;19) chromosome translocation in *Gorilla gorilla*, suggesting a potential role for transpositions in evolution of the primate genome. Thus, a series of consecutive segmental duplication events during primate evolution resulted in complex genome architecture in proximal 17p. Some of the more recent events led to the creation of novel genes that in human are expressed primarily in the brain. Our observations support the contention that serial segmental duplication events orchestrated primate speciation both by the generation of novel fusion/fission genes as well as by genomic inversion associated with decreased recombination rates further facilitating gene divergence.

Haplotype structure and recombination in *CAPN10*, a diabetes candidate gene. *V.J. Clark¹, J. Vander Molen¹, M. Hammond¹, A. Stone², A. Di Rienzo¹.* 1) Department of Human Genetics, University of Chicago, Chicago, IL; 2) Department of Anthropology, Arizona State University, Tempe, AZ.

It has been proposed that *CAPN10* variation contributes to the risk of type 2 diabetes. The entire *CAPN10* gene was previously re-sequenced in four population samples (Italians, the Hausa of Cameroon, Han Chinese, and Mazatecos from Mexico) in our laboratory. This study revealed a 4 kb region with an excess of polymorphism levels relative to divergence and relative to genome-wide levels of variation. To elucidate the relevance of this unusual genomic region to type 2 diabetes susceptibility, several aspects of nucleotide variation [patterns of polymorphism, haplotype structure, and linkage disequilibrium (LD)] were assessed by re-sequencing an additional set of Mexican-American type 2 diabetes cases and controls. Network analysis of the case-control and population sequence data reveals distinct patterns of haplotype structure between the Hausa (native Africans) and the non-African population samples. Further analysis of SNP haplotypes indicates 5 haplotype clusters common among non-Africans. With this analysis we were able to refine previously identified type 2 diabetes risk haplotypes composed of other SNPs in *CAPN10* (*i. e.* UCSNP-43, -19, and -63) in multiple populations and Mexican-American cases and controls. To complement the haplotype analyses, we are exploring the contribution of increased local rates recombination (a recombination hotspot) to the accumulation of polymorphism in this region. Sliding-window analysis of recombination (summarized by the H_{01} estimator of 4Nr) across the *CAPN10* gene indicates lower levels of LD coinciding with the peak of polymorphism; this pattern is most distinct in the Hausa. Analyses of sequences from 15 Western chimpanzees show, unlike in human populations, no local increase of either polymorphism levels or rate of recombination. Therefore, it appears that changes in mutation and recombination rates have occurred after the split of the human and chimpanzee lineages. We anticipate that this multi-faceted approach to explore patterns of variation in *CAPN10* will inform the design of future type 2 diabetes case/control association analyses.

Methods for improving haplotype reconstruction with microsatellite loci. *J.B. Ekins¹, L.A.D. Hutchison^{1,2}, J.E. Ekins¹, N.M. Myres¹, K. Hadley¹, L. Layton¹, M.L. Lunt¹, S.S. Masek¹, A.A. Nelson¹, M.E. Nelson¹, K.L. Pennington¹, U.A. Perego¹, J.L. Peterson¹, A. Sims¹, T. Tolley¹, A. Welch¹, S.R. Woodward^{1,3}.* 1) Sorenson Molecular Genealogy Foundation, Salt Lake City, UT; 2) Department of Computer Science, Brigham Young University, Provo, UT; 3) Department of Micro and Molecular Biology, Brigham Young University, Provo, UT.

Derivation of haplotype information from unphased genotypic data is vital to many genetic and genealogical questions, including the reconstruction of population history. Microsatellite loci exhibit higher mutation rates than biallelic loci and thereby become good candidates for asking questions about recent population history. These rates contribute to higher levels of identical-by-state allele values and offer a reasonable explanation for the lower absolute success rates obtained for STR datasets by haplotype reconstruction algorithms like PHASE v.2.0.2 (when compared to SNP datasets). This difficulty is exacerbated by the low average density of STR loci in the genome (as compared with available SNP loci) which, generally speaking, increases the noise of recombination events between pairs of loci. Selection parameters such as measures of disequilibrium have been successfully used to improve success rates when applied to SNP datasets. Herein, selection parameters (e.g. LD) and informational parameters (e.g. the extent of population admixture) are shown to improve haplotyping success rates in datasets containing linked STR loci. In addition to the use of simulated datasets (such as phase known diplotypes produced by combining haploid data), phase reconstruction trials using large, real STR datasets of phase-known diploid individuals are performed for the first time-- answering the need to establish success rates within real datasets composed of microsatellite loci. We also present a new haplotype reconstruction algorithm that iteratively infers population haplotype prevalences and haplotype phase probabilities for each sample. Our algorithm provides for the novel use of prior knowledge about individual-level admixture, and operates $\sim 6 \times 10^3$ times faster than PHASE, while achieving comparable levels of accuracy on equivalent datasets.

Variations in C3, C3a receptor and C5 genes affect susceptibility to bronchial asthma. *F. Kamada¹, K. Hasegawa², M. Tamari², M. Shimizu^{2, 3}, N. Takahashi², X.Q. Mao³, S. Doi⁴, H. Fujiwara⁴, A. Miyatake⁵, K. Fujita⁶, Y. Aoki¹, S. Kure¹, Y. Matsubara¹, T. Shirakawa^{2, 3}, Y. Suzuki¹.* 1) Department of Medical Genetics, Tohoku University School of Medicine, Sendai, Japan; 2) Laboratory for Genetics of Allergic Diseases, SNP Research Center, The Institute of Physical and Chemical Research (RIKEN), Yokohama, Japan; 3) Department of Health Promotion and Human Behavior, Kyoto University School of Public Health, Kyoto, Japan; 4) Osaka Prefectural Habikino Hospital, Osaka, Japan; 5) Miyatake Asthma Clinic, Osaka, Japan; 6) College of Nursing, University of Shiga, Shiga, Japan.

Bronchial asthma (BA) is a common chronic inflammatory disease characterized by hyperresponsive airways, mucus over-production, eosinophil activation and the production of IgE. The complement system plays an immunoregulatory role at the interface of innate and acquired immunity. Recent studies have provided evidence that C3, C3a receptor and C5 are linked to airway hyperresponsiveness. To determine whether genetic variations in the genes of the complement system affect susceptibility to BA, we screened single nucleotide polymorphisms (SNPs) in the C3, C5, C3a receptor (C3AR1), and C5a receptor (C5R1) genes and performed association studies in the Japanese population. The results of the single-SNP case-control study suggested an association between 4896C/T in the C3 gene with atopic childhood BA ($P=0.0078$) and adult BA ($P=0.010$). When patients were stratified according to high total IgE levels, 4896C/T was more closely associated with childhood and adult BA ($P=0.0057$ and $P=0.00057$, respectively). Furthermore, a patient-only association study suggested that severity of childhood BA was associated with 1526G/A of the C3AR1 gene ($P=0.0057$). We identified a high-risk haplotype of the C3 gene for childhood ($P=0.0021$) and adult BA ($P=0.0058$) and a low-risk haplotype for adult BA ($P=0.00011$). We also identified a haplotype of the C5 gene that was protective against childhood ($P=0.0000014$) and adult ($P=0.00063$) BA. These results suggest that the C3 and C5 pathways of the complement system play important roles in the pathogenesis of BA and that polymorphisms of these genes affect susceptibility to BA.

Portuguese and non-Portuguese origins of Machado-Joseph disease (MJD): a worldwide haplotype study. S.

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The name Machado-Joseph disease for a type of spinocerebellar ataxia resulted from the description of the disorder among the first families of Portuguese-Azorean origin. At present, this is the most common autosomal dominant ataxia in the world, affecting many different ethnic populations. Following a worldwide study, where two SNP-based haplotypes were found in families of Azorean extraction, we recently reported their uneven distribution in mainland Portugal. The ACA haplotype, specific to the Flores island, and shared by the vast majority of non-Portuguese families, is present in all patients from northeastern Portugal; on the other hand, the GGC haplotype, found in S. Miguel island and only in 7 non-Portuguese families, is mainly in the center and Tagus valley, accounting for 72.6% of the families.

We now studied 150 families from different origins with six intragenic SNPs. Additionally, and taking into account their higher mutation rate, four microsatellites, spanning less than 260 kb from the (CAG)_n, were assessed to unravel more about the recent history of these populations and, consequently, about the origins and possible routes of the mutations. Results from 89 Portuguese and 61 non-Portuguese families suggested either a very ancient origin of the TTACAC haplotype, or different mutational events spread worldwide. Nevertheless, the low microsatellite diversity of this MJD haplotype in Portugal suggests its recent introduction in our population. On the contrary, the GTGGCA lineage may be of Portuguese origin since it shows the highest molecular diversity among the patients harbouring this haplotype.

Program Nr: 1204 from the 2004 ASHG Annual Meeting

Database characterization of Y-chromosomal 39-locus haplotypes. *N.V. Myres¹, J.E. Ekins¹, K. Hadley¹, U.A. Perego¹, J.B. Ekins¹, L.A.D. Hutchison¹, L. Layton¹, M.L. Lunt¹, S.S. Masek¹, A.A. Nelson¹, M.E. Nelson¹, K.L. Pennington¹, J.L. Peterson¹, A. Sims¹, T. Tolley¹, A. Welch¹, S.R. Woodward^{1,2}.* 1) Sorenson Molec Genealogy Fndn, Salt Lake City, UT; 2) Dept. Microbiology, Brigham Young University, Provo, UT.

Forensic analysis based on Y-chromosomal STR loci relies on haplotype frequency estimates for assessing the significance of matching haplotypes. Current frequency estimates, accepted by the forensics community, are based on the minimal 9-locus and extended 11-locus haplotypes. To augment current estimates, the present study reports frequencies for the minimal and extended haplotypes for a dataset of 7,976 male samples collected in the United States, Canada, Europe, Oceania, South America, and Asia . These haplotypes are further extended to a total of 39 loci, for which frequencies are also reported. Additionally, up to 15 generations of paternal-line genealogy was collected for each male to provide a historical interpretation for observed haplotype stratification among populations, as measured by pairwise RST-statistics.

Allele at the *MRP1* gene locus shows evidence of positive selection in the Caucasian population. Z.H. Wang¹, K. Tang², E.J. Lee³, S.S. Chong^{4,5}, C.G. Lee^{2,6}. 1) Bioengineering Div, National Univ. of Singapore (NUS), Singapore; 2) Biochemistry Dept, NUS, Singapore; 3) Pharmacology Dept, NUS, Singapore; 4) Pediatrics, O&G Depts, NUS, Singapore; 5) Dept of Pediatrics, O&G, Inst Genetic Med JHU SOM, USA; 6) Medical Sciences Div, National Cancer Center, Singapore.

The multidrug resistance-associated protein 1, MRP1 (ABCC1) is a 190 kDa founding member of the C subfamily of the ATP binding cassette (ABC) superfamily of transport proteins. Like the multidrug transporter, MDR1, MRP1 effluxes similar spectrum of drugs and are commonly expressed in a large number of drug resistant malignancies. Hence, similar to the MDR1 transporter, single nucleotide polymorphisms (SNPs) within the *MRP1* gene may account for differences in drug response or disease susceptibility between different individuals. To facilitate association studies of this gene locus with complex diseases and drug response, we examined the haplotype and linkage disequilibrium (LD) profiles across the *MRP1* gene among the Chinese, Malay, Indian, Caucasian and African American populations. Thirteen SNPs spanning 186.4 kb of the *MRP1* locus were genotyped in 91-96 individuals from each ethnic population. Unlike the *MDR1* gene, the *MRP1* gene locus exhibited greater haplotype diversity and weaker LD. Nonetheless, despite its weak LD, a high frequency haplotype showed extended haplotype homozygosity across 135 kb in the Caucasian population. Notably, using coalescent simulations, statistically significant ($P < 0.05$) evidence of positive selection was observed for an allele contained in the high frequency haplotype in the Caucasian but not other populations under 4 different population models (constant size, expansion, bottleneck and structure) and 3 recombination rate assumptions (2.6, 1.3 and 0.65). Interestingly, alleles at the *MDR1* gene locus also demonstrated evidence of positive selection but in the Chinese and not the Caucasian population (Tang, et al 2004, Human Molecular Genetics 13:783-797). Taken together, these results suggest that different evolutionary pressures on different populations may result in the selection of different genes with similar functions in the different population.

Program Nr: 1206 from the 2004 ASHG Annual Meeting

A population genetic study of APOE and CYP2D6. *H. Payami, L. Moses, D. Yearout, M. Zhu, J. Montimurro, R. Keefe.* Wadsworth Ctr, NY Department Health, Albany, NY.

Association studies hold the greatest promise for dissecting genetics of complex disorders. Proper matching of cases and controls is thought to be essential, but in reality impractical. Most studies use the same controls for different patient populations, operating with the assumption that allele frequencies do not vary by age or sex. We question if unrecognized variations in control frequencies may be a reason why association studies are often irreproducible. The aim of this study was to assess genotype and allele frequencies of APOE and CYP2D6, two widely studied candidate genes, as a function of age and sex, and to test for deviations from Hardy Weinberg equilibrium (HW). We genotyped 1748 Caucasian individuals; binned data in 4 age groups: newborns (n=573), 20-49 yrs (n=279), 50-79 yrs (n=340), and 80+ yrs (n=556); and used Chi square for analysis. CYP2D6 frequencies deviated significantly from HW (p=.0001): *4 heterozygous was reduced, while both homozygous were increased. The same pattern (suggestive of heterozygous disadvantage) was seen in newborns (p=.03), which was an unbiased series of consecutive births, 20-49 yr group (p=.02), 80+ yr group (p=.003); in men (p=.005) and in women (p=.009). CYP2D6 frequencies did not vary significantly by age or sex. APOE findings were the opposite: all age groups were in HW, but there was significant genotype (p=.03) and allele frequency (p=.002) variation by age. This age effect was significant in women (p=.007) but not in men; and was driven primarily by the drop in e4 frequency in 80+ age group. In summary, CYP2D6 frequencies do not vary by age or sex, hence case-control matching for these variables is not critical. Departure from HW suggests the possibility of selective inferiority for the CYP2D6 mutant/wt heterozygous genotype. Sex and age matching is critical for APOE and can lead to spurious disease associations if ignored. We recommend that population genetic studies be performed routinely on candidate genes, before embarking on disease association studies. (Supported by NIH grants AG08017, NS36960, U24AG21886; and Oregon Newborn Screening).

Frequency of MTHFR C677T, MTHFR A1298C, Factor V Leiden G1691A and Prothrombin G2010A mutations in Systemic Lupus Erythematosus and normal mestizo Mexican populations. *M. Salazar-Paramo^{1,3}, I.P.*

Davalos^{2,3}, L. Gonzalez-Lopez⁴, L. Sandoval², L.E. Figuera^{2,3}, I.C. Valera^{1,3}, J.I. Gamez-Nava^{1,3}. 1) Unidad de Investigacion en Epidemiologia Clinica, IMSS; 2) Division de Genetica, CIBO, IMSS; 3) Departamento de Fisiologia, CUCS, Universidad de Guadalajara; 4) Departamento de Reumatologia, HGZ 110. Guadalajara, Jalisco, Mexico.

Systemic Lupus Erythematosus (SLE) is the prototype of autoimmune diseases. The prevalence of arterial and venous thrombotic events in SLE is high. Thrombophilia is an abnormal phenomenon related with increased formation of embolisms. Thrombophilic risk factors acquired and genetic (MTHFR C677T, MTHFR A1298C, FV G1691A and prothrombin G2010A mutations) represent a hypercoagulability risk. Objective: To determine the allelic (AF) and genotypic frequencies (GF) of MTHFR C677T, MTHFR A1298C, FV G1691A and prothrombin G2010A mutations in SLE and compared to a control group. Methods: The populations consisted of two groups: 1) Group SLE: mestizo Mexican patients with SLE (ACR1982) and 2) Group N: normal mestizo Mexican individuals. Groups were typed for the thrombophilic mutations by the PCR/RFLP method. Hardy-Weinberg Equilibrium was tested by chi square. Results: Group SLE, MTHFR C677T AF % (n): C 48(58), T 52(62), MTHFR A1298C: A 63(75), C 37(45). When analyzed combination of 1298AC/677CT genotypes in SLE group only 13% were 1298AA/677CC. FV G1691A: G 100(120), A 0(0), prothrombin G2010A: G 98(104), A 2(2). Group N, MTHFR C677T AF % (n): C 60(47), T 40(31), MTHFR A1298C: A 72(62), C 28(24), FV G1691A: G 98(84), A 2(2), prothrombin G2010A: G 96(92), A 4(4). Conclusions: AF differences between SLE and N group had not statistical significance. With respect to MTHFR C677T/A1298C, 87 % of the SLE had almost one mutated allele.

Reconciling estimates of linkage disequilibrium in the human genome. *Y. Chretien*^{1,2}, *I. Pe'er*^{2,3}, *P. de Bakker*^{1,2}, *M. Daly*^{2,3}, *D. Altshuler*^{1,2,4}. 1) Department of Molecular Biology and Diabetes Unit, Massachusetts General Hospital, Boston, MA; 2) Broad Institute of Harvard and MIT, Cambridge, MA; 3) Whitehead Institute of Biomedical Research, Cambridge, MA; 4) Department of Genetics and Medicine, Harvard Medical School, Boston, MA.

Patterns of LD in human populations determine the power of indirect association methods. Many large-scale surveys of LD have been performed, but the results of different studies have yielded inconsistent pictures of LD and the number of SNPs needed for LD mapping. We aimed to reconcile these previous reports by examining how descriptions of LD vary depending on attributes of the sampling scheme: SNP ascertainment, minor allele frequency spectrum, marker density, size of region examined, and the sample size in which allelic associations are defined.

We compared multiple characteristics of the data (pairwise measures of LD, haplotype blocks) across five large-scale, publicly available datasets: the HapMap ENCODE project [see abstract by Gabriel et. al.]; a series of genes resequenced by SeattleSNPs; a region of chromosome 20 studied by the Sanger Center; the Gabriel et al dataset published in 2002, and the genome-wide SNP data (>600,000 markers) from the HapMap project. To control for the effect of population origin, this report considers only datasets collected using the same European population (CEPH).

The five datasets appear quite dissimilar when taken at face value. These differences can largely be reconciled, however, based on the sampling schemes used by investigators. For example, the use of dbSNP and direct resequencing are largely similar as long as the frequency spectrum of SNPs identified is considered. These results provide a robust, consistent picture of LD across the human genome, and underscore the importance of understanding the biases inherent in the available approaches: characterizing modest sized regions and numbers of chromosomes by resequencing (as in SeattleSNPs) or sampling subsets of SNPs from the public SNP map (as in the HapMap data), and the different LD properties of SNPs in different allele frequency classes.

Evaluation of the Role of the Transition to Transversion Ratio on the Estimate of the Linkage Disequilibrium Coefficient. *J. Krushkal*¹, *E.F. Ryder*², *M. Pungliya*³. 1) Department of Preventive Medicine and Center of Genomics and Bioinformatics, University of Tennessee-Memphis, Memphis, TN; 2) Department of Biology and Biotechnology, Worcester Polytechnic Institute, Worcester, MA; 3) Genaissance Pharmaceuticals, Inc., New Haven, CT.

Genetic association studies attempt to identify genetic variants that show nonrandom associations with disease traits. A true, or non-spurious association of an allele at a polymorphic marker locus with disease is assumed to be caused by the linkage disequilibrium of the marker allele with a disease allele.

Because two biologically different types of nucleotide substitution, transitions and transversions, often occur at different rates, many previous studies have shown that for estimation of evolutionary distances among divergent sequences from different species it is important to properly evaluate the pattern of nucleotide substitution, i.e., the contribution of different types of nucleotide changes. In this work, we studied the effect of the differences between transitions and transversions on the association among alleles from sites of closely related sequences from the same species. We investigated the role of the transition/transversion ratio on the estimated value of linkage disequilibrium coefficient, D , between a pair of single nucleotide polymorphism (SNP) loci. We investigated the SNP frequency distribution and estimated the transition/transversion ratio among human SNPs using public SNP databases. A range of transition/transversion ratios and different recombination rates were then used to simulate sequence evolution in human populations. For every set of parameters studied, the mean estimated values of the linkage disequilibrium coefficient were independent of the transition/transversion ratio.

Program Nr: 1210 from the 2004 ASHG Annual Meeting

Patterns of genetic diversity, haplotype structure, and linkage disequilibrium at interleukin-4 (*IL-4*) and interleukin-13 (*IL-13*) in human populations. A. Ranciaro, E. Tarazona-Santos, S.A. Tishkoff. Biology, University of Maryland, College Park, MD.

IL-4 and *IL-13* are cytokines mapped to 5q31 involved in Th2 immune response against parasites such as *Plasmodium Falciparum* and helminthes. Because infectious disease has likely resulted in selective pressure during human evolutionary history, *IL-4* and *IL-13* are candidate genes to be targets of natural selection. They also play a role in susceptibility to common complex diseases of the immune system such as asthma and allergies. We have examined nucleotide variation in a 2.5 kb region of *IL-4* (including the promoter containing a SNP (-589 CT) associated with resistance to malarial infection and other infectious diseases) and 3.9 kb of *IL-13* (including the entire coding and promoter regions) in 280 individuals (*IL-4*) and 132 individuals (*IL-13*) from West Africa, East Africa, Asia, Europe and South America. We observed different patterns of nucleotide diversity, haplotype structure and linkage disequilibrium in African and non-African populations, and we identify a set of htSNPs from each geographic region that will be useful for functional genetic association studies of complex disease. We observe an excess of high frequency derived alleles in Europeans and East Africans at *IL-13* and high frequencies of two divergent haplotype clades at *IL-4* (each associated with different alleles at SNP -589). We use several statistical tests to distinguish the effects of natural selection and demographic history on patterns of genetic diversity at these loci. The results of this study will have important implications for identification of SNPs and haplotypes useful for gene-mapping studies of complex diseases, as well as for studies of genetic susceptibility to infectious diseases influenced by Th2 immune response, and the genetic basis of adaptation during human evolution. Funded by BWF and Packard Career awards, and NSF grant BCS-0196183 to ST.

Association of the NRAMP1 gene polymorphisms and susceptibility to tuberculosis in Mestizo population of Mexico. *L. Sandoval*¹, *M.C. Ruvalcaba Garcia*¹, *A. Corona Nakamura*², *G. Amaya Tapia*⁴, *L.E. Becerra Solano*^{1,3}, *L. Martin del Campo Plascencia*⁵, *M.C. Moran Moguel*^{1,3}, *T.P.K. Reddy*¹, *I.P. Davalos Rodriguez*^{1,3}, *L.E. Figuera*^{1,3}, *F. Rivas*³, *J.M. Cantu*³. 1) Divisiones de Genetica y Medicina Molecular, CIBO-IMSS, Guadalajara, Mexico; 2) Infectologia, HE, CMNO, IMSS, Guadalajara, Mexico; 3) Doctorado en Genetica Humana, Universidad de Guadalajara, Mexico; 4) Infectologia, Hospital General de Occidente, SSA, Guadalajara, Mexico; 5) LLARRE, IMSS, Guadalajara, Mexico.

Tuberculosis (TB) is a major cause of human mortality by infectious agents. It has been well established that the host genetic background is an important modulator of TB susceptibility. Several genes linked to TB susceptibility have recently been identified. These include NRAMP1, VDR, IFN-gR, HLA haplotypes, IL-10, IL-12 and their receptors. Genetic polymorphisms of NRAMP1 affect innate host resistance probably through the defective production and function of Nramp1, and the NRAMP1 gene has been associated with TB susceptibility in several ethnic groups. The aim of the present study was to determine whether NRAMP1 polymorphisms are associated with TB susceptibility in a Mestizo population of Northwest Mexico. A total of 56 pulmonary TB (PTB) patients, 27 extrapulmonary TB patients (ETB), and 73 healthy control subjects (C) were studied. Four NRAMP1 polymorphisms: a single nucleotide change in intron 4 (469 + 14G/C, INT4), a non-conservative single-base substitution at codon 543 (D543N), a TGTG deletion in the 3 untranslated region (1729 + 55del4, 3UTR) and a microsatellite GT repeat at 5 to exon 1 ([GT]n), were typed by PCR-RFLP. Genotype distribution in all three groups showed no deviation from Hardy-Weinberg equilibrium. Inter-group comparison yielded no significant difference for allele and genotype distributions at regions INT4, D543N, (GT)n. Only allele 3UTR del were found increased in the PTB group (16.1%) when compared to group C (6.8%, $X^2 = 5.57$; $p = 0.02$). These results support an association between NRAMP1 3UTR polymorphism and PTB.

Different patterns of Linkage Disequilibrium (LD) among Corsica sub-populations. *G. Sole*¹, *V. Latini*¹, *L. Varesi*², *M. Memmi*², *G. Vona*³, *A. Cao*¹, *M.S. Ristaldi*¹. 1) INN-CNR, Selargius (CA), CA, Italy; 2) University of Corte, France; 3) University of Cagliari, Italy.

Genetic isolates with a history of a small founder population, long-lasting isolation and population bottlenecks represent exceptional resources in the identification of genes involved in the pathogenesis of multifactorial diseases. In these populations the disease allele reveals Linkage Disequilibrium (LD) with markers over significant genetic intervals, therefore facilitating disease locus identification. This study has been designed to examine the amount and decay of LD with distance in some sub-populations of Corsica: Corte, Niolo, Bozio, (located in the mountainous north-center of the island) and Ajaccio (located in the south west of the island in a stretch of coast area). Furthermore LD has been analyzed in the general population of Corsica in order to assess the average LD extension in the island. We have analyzed the LD extension on the Xq13.3 genomic region that has been widely used as a measure of general LD in a given population and to compare the levels of LD between populations. We have found an extreme variability of amount of LD among the different populations. Bozio and Niolo show an high degree of LD while Corte, Ajaccio and the general population of Corsica show low degree of LD. The same variability characterizes the pattern of disequilibrium: distance can explain from less than 2% up to 50% of the total LD variation in relation with the different populations. These results are likely due to a different genetic structure in these isolates (even if geographically neighbouring). Number of founders, expansion rate, amount of immigration are factors that could affect the observed micro differentiation and LD extension. Our results stress the importance of choosing the proper sub-populations to carry out studies aimed to the identification of genes involved in complex diseases, because differences in demographic history of the isolated populations demand different strategies.

Allele frequencies of seven short tandem repeat loci in the northern Iranian population. A. Ghamari¹, B. Moghimi², S.S. Hosseini³, H. Najmabadi³. 1) Genetics research center, Social welfare Univ., Tehran, Tehran, Iran; 2) Medical department, university of Tehran; 3) Kariminejad Najmabad Pat. Genetics Center.

The short tandem repeat (STR) loci are highly polymorphic markers, which are of major importance in the field of linkage studies, gene mapping and DNA fingerprinting. To be useful in these areas, any marker must display a significant degree of polymorphism. We studied the informativity of seven of these markers in the northern Iranian population. DNA was extracted from blood samples of 50 normal unrelated individuals. Seven different loci namely, TPOX, HUMTH01, F13B, D16S539, D13S317, D8S1179, and D5S818 were amplified by PCR for each DNA sample. Polyacrylamide gel electrophoresis of PCR products and home-made allelic ladders was carried out, followed by allele frequency and heterozygosity rate determination for each marker. The heterozygosity of markers were calculated as follows: D16S539: 0.812, D13S317: 0.7876, D8S1179: 0.7992, D5S818: 0.761, HUMTH01: 0.7842, F13B: 0.7614, and TPOX: 0.6634. These data offer D16S539 as the most informative marker in the northern Iranian population, followed by D8S1179, D13S317, HUMTH01, F13B, D5S818, and TPOX, respectively. The heterozygosity of about 0.7 for all of markers guarantees using them in linkage studies, gene mapping and DNA fingerprinting.

A single nucleotide polymorphism at the perilipin gene associated with reduced body fat in the Newfoundland population. *G. Sun, C. French, S. Cooke, G. Martin, A. Ma, A. Kennedy, H. Zhang.* Genetics, Memorial Uni. of Newfoundland, St. John's, Newfoundland, Canada.

Perilipin is a hormone-regulated phosphoprotein that coats the surface of lipid droplets in adipocytes and plays an important role in the regulation of human adipocyte lipolysis. The present study was designed to investigate whether a single nucleotide polymorphism (SNP) in the perilipin gene associates with body composition in a large population. Six hundreds and twelve subjects (105 men and 507 women aged 19 to 60) were recruited from the genetically homogeneous population of the Canadian province of Newfoundland and Labrador (NL). Subjects were healthy and at least a third generation resident of NL. Percent body fat (%FAT), percent trunk fat (%FAT-T), lean body mass (LBM) and fat mass (FAT) were measured using dual-energy X-ray absorptiometry. Genomic DNA was extracted from whole blood. An adenine to guanine substitute (A/G) SNP located in the 3rd intron of the perilipin gene was genotyped using TaqMan technology (Assay-On-Demand SNP genotyping kit). A chi square analysis was performed to determine Hardy-Weinberg equilibrium. Association analysis was performed using SPSS software. The frequency of the minor G allele was significantly higher in subjects whose %FAT-T <28% than those whose %FAT-T > 38% (0.32 vs. 0.25; $p < 0.05$ by chi square test). Moreover, %FAT-T was compared among GG, AG and AA genotype groups. GG homozygous subjects showed the least (33.0%), followed by AG heterozygous (36.3%) and AA homozygous subjects with the highest %FAT-T (36.6%) ($p < 0.05$ by ANOVA adjusted for age and gender, and corrected by Bonferroni). Our study showed that the minor G allele associates with significantly less %FAT-T. The same trend was observed for %FAT but was not statistically significant. This is the first study to show that a polymorphism in the perilipin gene is associated with the variation of central obesity at the population level.

Program Nr: 1215 from the 2004 ASHG Annual Meeting

High-throughput resequencing of mitochondrial DNA from major human haplogroups using base-specific cleavage and MALDI TOF mass spectrometry analysis. *M. Ehrich¹, M. Nelson², J. Turner¹, M. Langdown², D. van den Boom¹.* 1) Dept Molecular Biology, SEQUENOM Inc., San Diego, CA; 2) Dept Biostatistics, Sequenom Inc., San Diego, Ca.

Many studies have attempted to link variations in the mitochondrial genome to various diseases with an excess of maternal inheritance. However, to date, few common variations with measurable effects have been identified. One possible reason for the lack of success may be the absence of common variations responsible for the mitochondrial component of disease susceptibility. If rare variations are responsible for the mitochondrial contribution to disease risk, this could only be identified and verified by complete sequence assessment. Here we present a novel application using base-specific cleavage and MALDI TOF mass spectrometry analysis that enables high-throughput mitochondrial resequencing. We established a set of 20 PCR amplicons to analyze the complete mitochondrial genome of 186 individuals in a case-control study. Each group contained 88 individuals from the common European H/V haplogroup and one individual from each of the other common haplogroups (J, U, T, A, I). We found a total of 281 SNPs in all samples. Currently, 120 of the identified SNPs are not listed in the Mitomap database. Within the H/V haplogroup, the vast majority of genetic variations are rare, occurring only in one or two subjects. It is noteworthy that two scientists carried out the sequencing effort in a single day.

Program Nr: 1216 from the 2004 ASHG Annual Meeting

Mitochondrial DNA Data Analysis Using Applied Biosystems SeqScape Software v2.1.1. *A. Pradhan, Q. Doan, J. Sorenson, R. Fang, C. Forbes, S. Vijaychander, R. Nutter, S. Nidtha, P. Baybayan.* Applied Biosystems, Foster City, CA.

Sequence variations in mitochondrial DNA have been used to study human evolution, forensic examinations and disease association studies such as Parkinsons and Alzheimers disease. Resequencing of the genome is often carried out using homebrewed methods, while data analysis continue to be a major bottleneck. We present here an integrated solution to resequencing of mitochondrial DNA using Applied Biosystems VariantSEQR Resequencing System and SeqScape Software v2.1.1. This system solution provides researchers the convenience of accurate variant identification with simplified data analysis and reduces the time required for many laborious and repetitive steps. Applied Biosystems SeqScape software v2.1.1 was designed specifically to address the needs of analyzing and identifying sequence variations accurately and reliably, which are critical requirements for resequencing applications such as mutation detection and analysis, SNP discovery and validation, pathogen sub-typing, allele identification, and sequence confirmation. The VariantSEQR Resequencing System includes resequencing sets with predicted confidence values, robust protocols to streamline experimental procedures and a SeqScape Software project template. This project template includes the relevant reference sequence, recommended analysis settings and can be used multiple times. Data analysis using this template can be done with a single click to provide basecalling, sequence assembly, alignment, sequence comparisons and variant identification. We illustrate here analysis of resequencing data from four Coriell DNA Samples for the mitochondrial genome using SeqScape Software v2.1.1. Results could be viewed in comprehensive reports, which contained mapping of each variant in the context of the entire mitochondrial genome and detection of all types of mutations such as insertions, deletions, substitutions and heterozygous indels with quality values. SeqScape Software v2.1.1 resulted in accurate variant identification, less manual data manipulation and shorter turnaround time for the complete analysis of the mitochondrial resequencing project.

The Spectrum of Mitochondrial DNA Mutations in Iranian Patients With Lebers Hereditary Optic Neuropathy.

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We studied 14 patients with Leber's hereditary optic neuropathy (LHON) to investigate the mtDNA haplotypes associated with the primary mutation(s). Eleven patients carried the mitochondrial DNA (mtDNA) G11778A mutation, while one had the T14484C mutation; one patient had the G3460A mutation and one the G14459A mutation. The Iranian G11778A LHON mutation was not associated with two mtDNA haplogroups M (0.0% compared with 3.2% in healthy controls) and J (7.7% compared with 10% in healthy controls). Our results showed a similarity in the pattern of LHON primary point mutations between Iranian families with LHON and those of Russian, European, and North American origin. Our results also do not support an association between mtDNA haplogroups J and M with LHON primary point mutations.

The evolutionary history of the dystrophins and dystrobrevins. *R.G. Roberts¹, S. Tan¹, H. Jin¹, M.J. Greener²*. 1) Division of Medical & Molecular Genetics, King's College London, Guy's Hospital, London, United Kingdom; 2) Institut de Génétique Humaine, CNRS - UPR 1142, 34396 Montpellier, France.

The dystrophins and dystrobrevins constitute a family of five proteins in all vertebrates. Members of the dystrophin sub-family (dystrophin, utrophin and DRP2) heterodimerise with members of the dystrobrevin sub-family (- and - dystrobrevin). Both are critical for the assembly and maintenance of dystrophin glycoprotein complexes (DGCs), the absence of which causes Duchenne muscular dystrophy (DMD), a lethal X-linked genetic disorder. The function of the DGC and the mechanism of DMD are essentially still unknown.

By tracing the elaboration of the gene family through evolutionary history, we have found genes encoding a single dystrophin and a single dystrobrevin in all invertebrate organisms hitherto examined, and have continued to search for additional orthologues in organisms which represent critical turning points in evolution. These include the identification of a single common ancestor of dystrophin and utrophin (the two most closely related vertebrate dystrophins) in cyclostomes (lampreys and hagfish), and the pursuit of ever more divergent dystrophins and dystrobrevins in more distantly related metazoans (cnidarians, ctenophora and porifera), converging on extant orthologues of the single last common ancestor of dystrophins and dystrobrevins. It is presumed that this latter molecule, the ur-dystrophin, existed as a homodimer.

The work includes alignments of over 60 dystrophin and dystrobrevin sequences, culled from degenerate RT-PCR of marine biological samples and bioinformatic sources. They are analysed in terms of patterns of conservation (with reference to the crystal structure of the cysteine-rich domain of dystrophin), the order of acquisition or loss of structural features (such as conditional loss of the C-terminal amphipathic helix and modulation of syntrophin stoichiometry by insertion of alternatively spliced exons), and the position of known pathogenic mutations.

Hereditary hemochromatosis in Sao Miguel island (Azores): A population and clinical approach. *L. de Fez, R. Cabral, C.C. Branco, P.R. Pacheco, L. Mota-Vieira.* Molec Genet & Pathol Unit, Hosp Divino Espirito Santo, Azores Islands, and Instituto Gulbenkian de Ciencia, Oeiras, Portugal.

The knowledge of the type, frequency and distribution of recessive mutations is very important in populations living in small geographic areas. Here we studied the hereditary hemochromatosis (HH, OMIM 235200), a recessive and iron overload disease mainly caused by mutations in the *HFE* gene, in the population of Sao Miguel island, the biggest (746.79 km²) and the most populated (131,609 inhabitants) island of the Azores (Portugal). Our approach is based on a screening by a PCR-RFLP of the 3 principal *HFE* mutations (C282Y, H63D and S65C) in a control population of 203 unrelated blood donors, representing the 6 municipalities of the island, and in 14 HH patients with elevated transferrin saturation levels (TS>40%). In the control group, we observed allele frequencies similar to those found in Central Europe: 4.93% for C282Y, 21.67% for H63D, and 1.97% for S65C. Moreover, nine genotypes were identified: wild/wild (50.74%), wild/H63D (31.03%), wild/C282Y (7.88%), H63D/H63D (4.93%), wild/S65C (2.96%), C282Y/H63D (0.99%), and C282Y/C282Y, H63D/S65C, H63D-S65C/H63D all with a frequency of 0.49%. We also investigated the geographic distribution of the mutant alleles within the island. Only for C282Y, the more severe mutation, we observed a statistically significant difference between East (9.3%) and West (3.6%). The molecular analysis of the 14 HH patients revealed no more than 3 subjects with the C282Y mutation: 2 were C282Y homozygotes and 1 was C282Y heterozygote. Of the remaining 11 patients, 1 was H63D/S65C compound heterozygote, 3 were H63D heterozygotes, and 7 have no characteristic *HFE* mutations. In the non-C282Y patient's group, we excluded the presence of a new mutation in this codon by DNA sequencing. In order to investigate the cause of iron overloading in these patients, we are analysing the recessive E60X, M172K and Y250X mutations in the *TFR2* gene, which is involved in iron homeostasis. In conclusion, our data indicate that the molecular basis of hereditary hemochromatosis in Sao Miguel's population is more complex than expected. Funded by DRCT. (lmotavieira@hdes.pt).

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Non-Paternity and Locus Specific Mutation Rates of 36 Y Chromosome STRs. *K. Hadley¹, N.M. Myres¹, J.B. Ekins¹, J.E. Ekins¹, L.A.D. Hutchinson^{1,2}, L. Layton¹, M.L. Lunt¹, S.S. Masek¹, A.A. Nelson¹, M.E. Nelson¹, K.L. Pennington¹, U.A. Perego¹, J.L. Peterson¹, A. Sims¹, T. Tolley¹, A. Welch¹, S. Woodward^{1,3}.* 1) Sorenson Molecular Genealogy Foundation, Salt Lake City, UT; 2) Computer Science, Brigham Young University, Provo, UT; 3) Department of Micro and Molecular Biology, Brigham Young University, Provo, UT.

Mutations and non-paternities are two of the most influential factors that may bias conclusions in Y-chromosome (y-ch) population genetic and lineage studies. Previous studies have determined locus specific mutation rates for 12 y-ch loci. Herein, we characterize mutation rates of 36 STR loci based on 500 individuals separated by between 1-13 meioses, including eight previously reported loci for comparison to published rates. Non-paternity rates have been shown to vary among certain demographics, age groups, and time periods. To account for all of these factors, the present study reports a non-paternity rate for samples collected from over 100 different families that vary in age group, culture, country of origin and the number of meioses separating them.

Sequence variation and haplotype diversity at the *CYP3A* locus. *E.E. Thompson*¹, *H. Kuttab-Boulos*², *D. Witonsky*², *B. Roe*³, *A. Di Rienzo*². 1) Committee on Genetics, University of Chicago, Chicago, IL; 2) Department of Human Genetics, University of Chicago, Chicago, IL; 3) Advanced Center for Genome Technology, University of Oklahoma, Norman, OK.

Members of the cytochrome P450 3A subfamily play important roles in the metabolism and clearance of endogenous substrates, such as bile acids and hormones, environmental compounds, and prescribed drugs. Two enzymes in particular, *CYP3A4* and *CYP3A5*, are involved in substantial first pass drug metabolism due to high expression levels in the liver, kidney and intestine. Both enzymes are characterized by genetic polymorphisms with high inter-ethnic variation, but the observed range of phenotypic response to *CYP3A* substrates is not completely explained by the known genetic data. In addition, few variants have been consistently reported to be associated with specific phenotypes, which may be due in part to large differences in allele frequencies across populations contributing to inconsistent results from association studies. We surveyed sequence variation at *CYP3A4* and *CYP3A5* in three ethnically diverse population samples in order to identify additional polymorphisms, describe patterns of linkage disequilibrium and haplotype structure, and investigate the role of natural selection at the *CYP3A* locus. We used a combination of comparative genomics and computational predictions to identify conserved noncoding sequences (CNS) and clusters of liver-specific transcription factor binding sites. Our results show large allele frequency differences between African and non-African populations, with a defined haplotype structure and strong LD extending across the 150 kb spanned by these two genes. Low levels of nucleotide diversity combined with a high frequency of derived alleles in one haplotype found at high frequency outside Africa are consistent with the effects of a partial selective sweep. Genotyping of a functional allele that defines this common haplotype (*CYP3A5*3*) reveals a highly unusual geographic distribution, again consistent with the action of population-specific selective pressures. The implications of these results for association studies of *CYP3A* variation will also be presented.

3 HAPLOTYPES OF THE α -GLOBIN GENE IN α -CHROMOSOMES OF MEXICAN INDIVIDUALS. *B. Ibarra, M.T. Magaña, F.J. Perea, Z. Ongay.* División de Genética, Centro de Investigación Biomédica de Occidente, Guadalajara Jalisco, México.

α -globin gene family have shown a high polymorphic diversity organized in 5 and 3 haplotypes (5 and 3 Hp). α -chromosomes (α -chr) are in linkage disequilibrium (LD) with 5Hps Bantu, Benin, Senegal, Cameroon and Arab-Indian. In the west coast of Mexico, in Mexican mestizos with African roots, we observed the following 5Hps in α -chr, Bantu (78.8%), Benin (18.2%) and atypical 9 (3.0%). With the purpose to establish the 3Hp, we analyzed 38 DNA polymorphic sites (six by RFLPs and 32 by DNA sequencing) in 33 unrelated α -chr (28 Bantu, four Benin and one atypical 9). The polymorphic sites were structured according to Harding et al (1997) and Lapoumeroulie et al (1992). All Bantu α -chr showed 13A1 3Hp with (AT)₆T₉ repeats (84.9%), a new 3Hp; the Benin were 2B2 (AT)₈T₄ (12.1%) and the atypical 9 4B1 (AT)₈T₅ (3.0%). Because of the high LD observed with Bantu and 13A1 Hps, we expect that if there is a single origin of Bantu α mutation, all of them must show at 3Hp the 13A1 polymorphic DNA sequence. A correlation between 5Hp and 3Hp could be observed with the other α mutations. The atypical 9 was also atypical at 3Hp with the repeats observed with the Cameroon α mutation, however it is different in one position with the typical Lapoumeroulie Cameroon Hp, therefore we can explain this α -chr by different genetic mechanisms or by a new α mutation. We stress the importance to study the DNA polymorphisms at 3Hp, to know the genetic diversity of α -chr as well as its implications in the α -gene expression and its possible effect in the clinical phenotype.

Screening of the single nucleotide polymorphisms in the protamine 1 and 2 genes of infertile men. *H.-S. Lee¹, Y.J. Jeong^{1, 2}, H.W. Choi¹, Y.-S. Park¹, S.Y. Park³, J.T. Seo⁴, M.K. Koong⁵, J.H. Jun¹.* 1) Infertility Research Lab., Samsung Cheil Hospital, Seoul, Korea; 2) Dept. of Animal Sciences, Chunbuk National University, Cheongju, Chungbuk, Korea; 3) Lab. of Medical Genetics, Samsung Cheil Hospital, Seoul, Korea; 4) Dept. of Urology, Samsung Cheil Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea; 5) Dept. of Ob/Gyn, Samsung Cheil Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea.

During spermatogenesis, a marked rearrangement in the sperm nucleus takes place involving the removal of histones and replacement by the highly positively charged protamine 1 and 2 (PRM1 and PRM2). These protamines are the most abundant nuclear proteins in the sperm nucleus packaging the male genome. The male infertility by haploinsufficiency of PRM1 or PRM2 has been reported in mouse model. The aim of this study is to identify the single nucleotide polymorphisms (SNPs) of PRM1 and PRM2 in the infertile men. Genomic DNA was extracted from peripheral bloods of infertile men with oligozoospermia or azoospermia, and analyzed using PCR and direct sequencing. We carried out the direct sequencing analysis of amplified fragments in PRM1 (557 nucleotides from -42 to 515) and PRM2 (599 nucleotides from 49 to 648) genes, respectively. Three SNPs of coding region in the PRM1 gene was found in the analysis of 130 infertile men. However, the SNPs at a133g (aa 96.9%, ag 3.1% and gg 0.0%), c160a (cc 99.2%, ca 0.8% and aa 0.0%) and c321a (cc 56.9%, ca 35.4% and aa 7.7%) coded the same amino acids, in terms of silence phenotypes. On the other hand, as results of the PRM2 gene sequencing in 164 infertile men, only two SNPs, g398c (gg 62.2%, gc 31.1% and ga 6.7%) and a473c (aa 63.4%, ac 29.9% and cc 6.7%), were identified in the intron of the PRM2 gene. We could not detect any SNP, which cause amino acid changes within the PRM1 and PRM2 genes. These results suggest that the PRM1 and PRM2 genes are highly conserved and essential for infertile men as well as normal male. We are trying to elucidate the genetic factors and causative genes, which should be implicated in human male infertility.

Sequence variation and intron 10 mRNA retention at human *NXF1*, an orthologue of a mouse modifier of retrovirus mutations. G. Wen, D. Concepcion, B.A. Hamilton. Dept Medicine/Division of Genetics, Univ California, San Diego, La Jolla, CA.

NXF1 encodes an essential component of the mRNA nuclear export machinery. Our lab has recently shown that a common variant in mouse *Nxf1* acts as a genetic modifier of mutations caused by a class of retrovirus insertions. To ask whether human alleles of *NXF1* might similarly respond to molecular parasites, we have examined sequence and expression polymorphism in *NXF1* and used evolutionary comparisons to predict a potential novel auto-regulatory mechanism. We sequenced conserved regions of *NXF1* from 117 anonymous subjects representing 13 different populations from the NIGMS human diversity collection. Across a 9289 bp footprint, we identified 24 variant sites, but only 5 with minor allele frequencies greater than 5% in this global sample. The nucleotide diversity (π) of *NXF1* is substantially lower than that for other genes in this or similar populations. Standard tests for neutrality, site-frequency spectrum and comparison between human and chimp sequences showed significant departure from neutrality for introns, which contain most of the polymorphic sites. We also examined allelic expression differences in heterozygous cell lines carrying a single SNP in the encoded RNA (cSNP). Using multiple assays, we find haplotype-specific expression differences of up to 60%. Surprisingly, EST data shows an unexpectedly high level of clones that either include part or all of highly conserved intron 10. RT-PCR and northern blots showed that the entire intron 10, but not other introns, is retained in a significant pool of polyadenylated RNA. RNP immunoprecipitation using anti-NXF1 antibodies shows mRNA containing intron 10 binds to export machinery. However, western blots do not show a protein of the predicted size translated from intron 10-containing mRNA. In conclusion, our data show surprisingly little nucleotide variation in human *NXF1*, but significant allelic variation in RNA expression level among common haplotypes. Test of neutrality suggest that *NXF1* might be subject to positive selection. It is likely that intron 10 retention of mRNA plays a role in self-regulation.

X-chromosome Polymorphisms and the Genetic Structure of Amerindian Populations. *S. Bourgeois¹, V. Yotova¹, A. Lovell¹, F. Xiao¹, C. Pich¹, C. Moreau¹, S. Bourthoumieu¹, E. Zietkiewicz¹, R. Michalski², A. Ruiz-Linares³, D. Labuda¹.* 1) Sainte-Justine Hospital, Montreal, PQ, Canada; 2) Victoria Hospital, Prince Albert, SK, Canada; 3) The Galton Laboratory, University College, London, UK.

Genetic data influencing the debate on the peopling of the New World and the place of Native Americans among other populations are essentially limited to these on the non-recombining portion of the Y chromosome and the mitochondrial DNA. There is a lack of similarly extensive studies of neutral nuclear non-Y-chromosome polymorphisms that could broaden the discussion and bring arguments unrestricted by paternal only or maternal only transmission. Toward this goal, we report data on the X-chromosome haplotype consisting of bi-allelic polymorphisms and a microsatellite within an 8 kb segment of the dystrophin gene, for 12 Amerindian populations (n=414 chromosomes) six Asian (n=668), six European (n=463) and three (n=130) of mixed European/Amerindian origin. Among twenty different haplotypes found in Native American populations, four represented 94% of all chromosomes from this continent. Three other haplotypes (representing less than 1%) were specific for Amerindians. Noticeable heterogeneity in haplotype frequencies among Amerindian populations correlates with the geographical localization and distance (Mantel test) but not with the linguistic grouping. Two haplotypes frequent in Eurasia were virtually absent in the Americas, whereas the occurrence of two others was significantly increased in Amerindian populations, consistent with a model of single entry during colonization of these continents. Continental distribution of the haplotypes, also when analyzed by PCA and pairwise FST, indicates a privileged link between the Amerindian, Siberian and Western European populations, rather than between the Amerindian and East-Asian populations. This link is not due to a recent admixture, but rather reflects a common ancestry of these populations. The migration towards the New World could have started in Northern Asia, and subsequently given rise to both NaDene and Amerind speaking people, following diversification of these populations. (Supported by the Canadian Institutes of Health Research MOP-67150).

BAT-25 and BAT-26 microsatellite profile in a sample of individuals of African origin from Rio Grande do Sul, Brazil. *S.L. Cossio*¹, *R.S. Coura*^{2,3}, *M.C. Bortolini*^{2,3}, *P. Ashton-Prolla*^{1,2,3}. 1) Medical Genetics Service, HCPA, Porto Alegre, RS, Brazil; 2) Department of Genetics UFRGS; 3) Post graduation course in genetics and molecular biology UFRGS.

Colorectal cancer (CRC) is one of the six most common cancers and, in Brazil, it is the third in mortality. Several clinical, pathological and morphological parameters are associated with disease prognosis. Molecular markers have also been associated with prognosis, especially in relation to therapeutic response and overall survival rates. Among these, microsatellite instability (MSI) is remarkable. It reflects genomic instability caused by a deficiency in the DNA repair system, which leads to the progressive accumulation of mutations, especially in mono- and dinucleotide microsatellites. Microsatellite stability (MS) status analysis is performed by the comparison between normal and tumoral tissues from the same patient and instability is characterized by the difference in the amplification profile of these tissues. MS status can be evaluated by a panel of five markers: BAT-25, BAT-26, D2S123, D5S346 and D17S250. The last three are polymorphic in several populations, but the former are considered quasi-monomorphic in populations of Caucasian origin. Thus, analysis of BAT25 and BAT 26 status in tumoral tissue only without comparison with the corresponding normal tissue has been considered sufficient for instability detection. One recent study however, showed high frequency of allelic variation for these two markers in a control population of african-american individuals. Our study describes the profile of BAT-25 and BAT-26 in a sample of 116 individuals of African origin from Rio Grande do Sul, Brazil. MSI analysis was performed by PCR and SSCP of 116 samples for BAT-25 and 110 for BAT-26. Allelic variation was 6.9% and 4.5% for BAT-25 and BAT-26, respectively. None of the samples presented simultaneous allelic variation in both markers. These results suggest a polymorphic profile of these markers in the studied sample and reinforce the importance of a comparative study between tumoral and corresponding normal tissue to determine their stability status in CRC. Support: CNPq, CAPES.

Dissecting cis-regulatory variation in the human genome. *E.T. Dermitzakis¹, R. Lyle², M. Forrest¹, S. Deutsch², C. Gehrig², L. Parand², M. Gagnebin², S. Hunt¹, P. Deloukas¹, S.E. Antonarakis².* 1) Wellcome Trust Sanger Institute, Hinxton, United Kingdom; 2) Department of Genetic Medicine and Development, University of Geneva Medical School, Switzerland.

Extensive sequence variation is present in human populations, but only a fraction of this is of functional significance. One way in which sequence variation can be functionally active is by modulating the levels of gene expression. Natural variation in gene expression is suspected to be involved in the etiology of complex disorders, and is likely to serve as useful intermediate markers, providing a more direct link between sequence variation and phenotypic outcomes. We have investigated the extent and pattern of gene expression variation in two different sets of genes. First, we have interrogated by Taqman the expression levels of forty-one chromosome 21 genes, using lymphoblastoid cell lines from the CEPH family collection. We have observed that the majority of genes have significant heritability values and, based on quantitative linkage analysis we infer that about 25% of them harbour cis-regulatory variation. We have now extended our screening of transcript levels using Illumina technology in a larger set of 700 genes from all the ENCODE regions, the whole Hsa21 and a 10 Mb segment of Hsa20. We have observed extensive variation in gene expression in the majority of genes as well cases of significant covariation of genes within the same genomic region, suggesting the presence of functionally variable regulatory regions that regulate the expression of more than one gene. We are currently using SNP genotypes from the Hapmap project as well as new SNP genotypes obtained in our platform to perform association tests between transcript levels and cis nucleotide variation in CEPH lymphoblastoid cell lines in order to identify cis regulatory variants. Preliminary analysis suggests that we are able to identify functionally variable regulatory regions that may serve as good functional candidates for association with quantitative phenotypes and complex disorders in humans.

Estimating allele frequencies and inbreeding coefficients in K-allele models. *S.E. Hodge*^{1,2}, *P. Gorroochurn*². 1) NYSPI, Unit 24, Columbia Univ, New York, NY; 2) Division of Statistical Genetics, Department of Biostatistics, Columbia University, New York, NY.

We examine some of the methods of estimation of allele frequencies and inbreeding coefficients in a K-allele model. Whereas the maximum likelihood estimators of the allele frequencies are equal to their observed (or sample) values under Hardy-Weinberg equilibrium, we show that, surprisingly, this is not the case when Hardy-Weinberg equilibrium does not hold, except in the $K = 2$ allele case. We also present a least-squares way of looking at the estimation problem, which fits observed genotype counts to their expected values. We use simulations to compare the biases and standard deviations of the three estimators (sample, maximum likelihood, and least-squares) in a 3-allele model, and show that the maximum likelihood estimators become more precise than the sample estimators with increasing sample size. Finally, we use probability-generating functions to derive exact expressions for the biases of the sample and Nei's estimators of the inbreeding coefficient in a 2-allele model for any sample size. Although Nei's inbreeding coefficient is usually preferred over the sample inbreeding coefficient since both its numerator and denominator are unbiased, we show that the sample inbreeding coefficient can sometimes be less biased than Nei's.

Alternatives to the Wright-Fisher Model and robustness of Mitochondrial Eve dating. *M. Kimmel*¹, *K. Cyran*². 1) Department of Statistics, Rice University, Houston, TX, USA; 2) Institute of Computer Science, Silesian University of Technology, Gliwice, Poland.

Several models for calculating the distribution of coalescence time exist, however their applicability is limited to a narrow spectrum of population trajectories. Also, they often rely on assumptions about generation to generation sampling scheme, known to be unrealistic for many populations, including the modern human population. We present a simulation-based approach capable of dealing with different population history scenarios, including populations evolving stochastically with environmental impacts variable in time. This approach allows comparing the branching process model of O'Connell with a range of Wright-Fisher type models. We apply our approach to estimate the age of the most recent common female ancestor based on the genetic material from mitochondrial DNA obtained from contemporary humans and extracted from Neanderthal fossils. The results indicate that when Neanderthals are used as an outgroup, the stochastic models based on branching processes provide similar estimates to those obtained with phylogenetic analysis, therefore supporting each other. On the other hand estimates assuming deterministic population growth are considerably higher, indicating that the stochastic demographic effects present in the human population when it was of a relatively small size are not negligible also for inferences dealing with long term history.

Y-chromosomal haplotype variation among the Finns. *P. Lahermo*¹, *S. Koivumäki*^{2, 3}, *P. Sistonen*⁴, *K. Huoponen*³, *M-L. Savontaus*^{2, 3}. 1) Finnish Genome Center, Univ Helsinki, Helsinki, Finland; 2) Department of Biology, Laboratory of Genetics, University of Turku, Finland; 3) Department of Medical Genetics, University of Turku, Finland; 4) Finnish Red Cross Blood Transfusion Service, Helsinki, Finland.

Y chromosomal variation was analyzed in 537 unrelated men from eastern and western Finland. Origins of those men were defined on the basis of their grandparental birthplace. Geographical distribution of the samples and sample sizes were as follows: Western Finland n=231 (consisting of the sub-populations: Southern Ostrobothnia 58, Hame 50, Southwestern Finland 50, Swedish speaking Ostrobothnia 25, and Satakunta 48) and Eastern Finland n=306 (sub-populations: Northern Karelia 22, Southern Karelia 48, Northern Savo 107 and Northern Ostrobothnia 129). Six biallelic and six microsatellite Y-chromosomal markers were studied. Haplotypes were constructed on the basis of five SNP loci (Tat, M9, 92R7, SRY-1532, M17), a Y- chromosome specific Alu-insertion (YAP, DYS287), and six microsatellite loci (DYS19, DYS388, DYS390, DYS391, DYS392 and DYS393). Genetic distances and diversity distributions were calculated using the ARLEQUIN 2.001 software package. Interpopulation variability was estimated for each haplotype and subhaplotype through 2 contingency test. Maximum parsimony networks for SNP haplogroups were constructed and phylogenetic relationships between the microsatellite haplotypes were analyzed with Network 3.0. Principal component analysis was carried out on both SNP and microsatellite data. Both SNP and microsatellite networks showed bifurcated structures reflecting the geographical origins and history of the western and eastern sub-populations. Similar clustering was evident in allelic and haplotype frequencies and genetic distances, giving evidence in support of the theory of two different founder groups for the Finnish population. This study suggests that most of the Y-chromosomal variation found among the Finns originates from the common European gene pool, with some eastern, possibly Finno-Ugric components especially in the eastern sub-population of Finland.

Worldwide polymorphism in classical human major histocompatibility (HLA) genes. *D. Meyer*^{1,2}, *R. Single*^{2,3}, *S.J. Mack*^{4,5}, *A. Lancaster*², *M.P. Nelson*², *H. Erlich*^{4,5}, *M. Fernandez-Viña*⁶, *G. Thomson*². 1) Dept. of Biology, University of So Paulo, Brazil; 2) Dept. of Integrative Biology, University of California, Berkeley, CA; 3) Dept. of Medical Biostatistics, University of Vermont, Burlington, VT; 4) Roche Molecular Systems, Alameda, CA; 5) Childrens Hospital Oakland, Research Institute, Oakland, CA; 6) CW Bill Young/DoD Marrow Donor Program, Naval Medical Research Center, Bethesda, MD.

The classical genes of the human major histocompatibility complex are critical to the adaptive immune response. Various lines of evidence indicate that these genes experience balancing selection. Here we survey data on HLA polymorphism generated by over 30 laboratories that participated in the Anthropology component of the 13th International Histocompatibility Workshop. Our results pertain to the HLA-A, B, C, DRB1, DQB1, DQA1, DPB1, and DPA1 loci. Populations with significant deviations from Hardy-Weinberg proportions were not included in the analyses. We selected 34 populations with at least 40 individuals (median = 94) typed for three sets of loci (class I, DRB1 and DQB1, all class II). We found high values for expected heterozygosity, resulting from many HLA alleles found at intermediate frequencies. Heterozygosity values were highest in African populations, and lowest in indigenous American and Australian populations, consistent with findings for putatively neutral markers, indicating that demographic history has shaped allele frequencies for these selected loci. The interpopulation differentiation at HLA loci has values which are typical of neutral markers, indicating that a simple model of balancing selection acting homogeneously on all populations does not account adequately for the data. Moderate to high LD was observed for all locus pairs, and decayed over chromosomal distance out to approximately 1Mb (after which a consistent effect of distance on LD was not observed). Rankings of world regions based upon LD were consistent with results for neutral markers. These results indicate that the demographic history of these populations along with the effects of balancing selection and drift has shaped variation at these HLA loci.

Gene frequencies of three genetic polymorphisms in malarious patients in south of Iran. *M.B. Mobasheri^{1,2}, P. Amirshahi²*. 1) Medical Genetics Department, School Of Medicine, Tehran University Of Medical Sciences(TUMS), Tehran, Iran; 2) Department Of Human Genetics,School Of Public Health, TUMS, Tehran, Iran.

It is established that several genetic markers specially blood genetic polymorphisms plays critical role in susceptibility to several diseases. Malaria is the most well known natural selective agent in tropical areas which has selected and established some blood genetic markers such as some of the haemoglobins, specially hemoglobin S in sickle cell anemia. Of course gene frequencies of these polymorphic systems in tropical areas is higher than others. To study the association between Malaria and three blood genetic markers, Haptoglobin(HP), Acid phosphatase(ACP) and Esterase-D(ESD) we selected a total of 205 highly susceptible malarious patients and 197 healthy controls from the same ethnic group, inhabited in an malaria endemic area in south of Iran. Using starch gel electrophoresis, the different phenotypes of each systems were determined. The results were analysed using Woolf's test. Significant association were found only in ESD system, in which the ESD2-2 phenotype with frequency of 6.2% in controls and 2% in patients were observed. Gene frequencies of common genes in each system in patients(P) and controls(C) and their relative gene frequencies(RF) are as following: HP1:[P(0.2716), C(0.2775), RF=1.0217], HP2:[P(0.7284), C(0.7225), RF=0.9919], ACP-A:[P(0.3683), C 0.3782), RF=1.0269], ACP-B:[P(0.6268), C(0.6167), RF=0.9839], ACP-C:[P(0.0049), C(0.0051), RF=1.0408], ESD1:[P(0.8308), C(0.7658, RF=0.9218], ESD2:[P(0.1692), C(0.2342), RF=1.3842]. A population under Hardy-Weinberg equilibrium shows no changes in the gene pool. In our study, however the gene ESD-2 showed more resistancy against malaria infection, but the population of origin is under Hardy-Weinberg equilibrium condition. If Genes better able to produce mature offspring than those without them, the frequency of those genes will increase. This is simple expressing Darwin's natural selection. It results from differential mortality and/or differential fecundity. More studies is required to know more about ESD in other populations of tropical areas to know if it has selective advantage against malaria.

DETECTION OF NOVEL SNPs PROVIDES FURTHER INSIGHTS INTO Y CHROMOSOMAL VARIATION IN PAKISTAN. *A. Mohyuddin¹, Q. Ayub¹, S. Firasat¹, P.A. Underhill², C. Tyler-Smith³, S.Q. Mehdi¹.* 1) Biomedical and Genetic Engineering Laboratories, Islamabad, Pakistan; 2) Department of Genetics, Stanford University, Stanford, CA USA; 3) The Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK.

Biallelic polymorphisms on the Y chromosome have been extensively used to study the history, evolution and migrations patterns of world populations. In this study we screened 8.5 kb of DNA in a panel of 93 Pakistani and 2 African individuals belonging to different haplogroups for new Y-SNPs. Male specific PCR products were mixed with a reference sample and analyzed by DHPLC. Four novel Y SNPs (PK2-5) were identified in the Pakistani samples and one (PK1) in the African sample belonging to haplogroup A of the YCC phylogenetic tree. The ancestral state of each SNP was determined in two chimpanzee samples and various Pakistani ethnic groups using ARMS-PCR or RFLP-PCR. The C to A (PK1) polymorphism found in the haplogroup A sample was also present in four other haplogroup A samples screened. All these samples were derived for the M91 and M6 mutations placing it in the sub clade A2. A T to C (PK2) mutation detected in two Burusho and one Hazara sample belonging to haplogroup C, was present in all haplogroup C-M217 derived Y chromosomes from northern Pakistan and absent in southern Pakistan. The PK3 (T to C) SNP was detected only in haplogroup L Kalash individuals and represents a new branch L/-3 of the phylogenetic tree. The A to T (PK4) mutation was detected in four Pathan samples belonging to clade O. The C to T (PK5) transition found in two Burusho individuals represents a new branch, R1a1/-d, on the M17 background and seems to be a recurrent mutation as it was also detected in the two chimpanzee samples. The detection of several novel Y-SNPs that have been found in the Pakistani ethnic groups enabled us to further investigate extant human Y chromosomal variation within Pakistan. Microsatellite diversity in sub clade L3 in the Kalash population was used to determine the time to the most common recent ancestor (TMRCA) using BATWING. The median TMRCA (95% CI) of 3300 (1500-7900) YBP corresponds to the time of invasion of the Indo-Pak subcontinent by the Indo European-speaking tribes from Central Asia.

Genetic diversity among European settlers of the Gaspé Peninsula (Quebec). *C. Moreau¹, V. Yotova¹, D. Gehl¹, C. Cavier², D. Sinnott¹, P. de Knijff³, H. Vezina², D. Labuda¹.* 1) Research Ctr, St-Justine Hosp, Univ Montreal, Montreal, Quebec, Canada; 2) Dpartement des sciences humaines, Universit du Qubec Chicoutimi; 3) Dept of Human Genetics, Ctr of Human and Clinical Genetics, Leiden University Medical Ctr.

The population of Quebec with its well-documented demographic history is conducive to study the relationship between genetic characteristics and the demographic history of human populations. The Gasp Peninsula, on the south shore of the St-Lawrence river, was colonized in the 18th and 19th centuries. We sampled four groups, namely Acadians, French-Canadians, Loyalists and descendents of settlers from the Channel Islands (Jerseyans), collecting DNA and reconstructing their genealogies. D-loop sequencing (n=368) demonstrated the presence of 112 distinct haplotypes, one of which accounts on 20% of the total sample (from 30% in Acadians to 13% in Jerseyans). Among European lineages we also find haplogroups A, C and D suggesting a significant Amerindian contribution. Y-chromosome lineages are dominated by haplogroups R1 and I, common in Europe, diversified into 132 microsatellite-based haplotypes, mostly singletons, with only few falling into higher frequency classes, especially among Acadians and Jerseyans. In turn, the X-chromosome genotyping reveals two chromosomes carrying Sub-Saharan African specific alleles. Again Acadians appear least varied with haplotype diversity of 0.780, compared to 0.793 for all and 0.814 for French-Canadian group. Two phenomena influenced genetic variability of these recent genetic isolates. The first one was the founder effect: related to the limited number of early settlers, it elevated the frequency of some rare alleles but was not strong enough to significantly change the frequency profile of common variants, which remained characteristic of the original European populations. Secondly, there were subsequent migrations, which, through the contribution of admixed founders of distinct European, or more easily detectable, Amerindian or even Sub-Saharan African origin, exerted a diversifying effect albeit of variable impact, on the populations of Gasp Peninsula. (Supported by NSERC and FRSQ).

Genetic history of Sao Miguel island (Azores): A HLA perspective. *P.R. Pacheco, A. Lismond, L. de Fez, R. Cabral, C.C. Branco, L. Mota-Vieira.* Molecular Genetics & Pathology Unit, Hospital Divino Espirito Santo, Azores Islands and Instituto Gulbenkian de Ciencia, Oeiras, Portugal.

Sao Miguel is the biggest (747 km²) and most populated island (131,609 inhabitants) of the Azores archipelago (Portugal). The island had no native population when the Portuguese first arrived in the XV century. It was peopled by the Portuguese, but Jews, Moorish, African slaves, Flemish, French and Spaniards also contributed to the initial settlement. To improve our knowledge of Sao Miguel population genetic diversity, we investigated the polymorphism of HLA-A, HLA-B and HLA-DRB1 loci. Blood samples were taken, after informed consent, from 106 unrelated blood donors, whose parents were born in Sao Miguel island. HLA typing was carried out using polymerase chain reaction with sequence specific primers (Olerup SSP HLA-A-B-DR SSP Combi Tray kit). Statistical analysis was performed with Arlequin v2.0. At the HLA-A locus, we identified 16 HLA-A alleles, of which A*02 (GF=0.2500), A*01 (GF=0.1509) and A*24 (GF=0.1368) are the most frequent. Of the 24 HLA-B alleles found, B*44 (GF=0.1557), B*08 (GF=0.1368) and B*14 (GF=0.0708) are the most prevalent. At HLA-DRB1 locus we found 13 alleles, of which the most prevalent are DRB1*07 and DRB1*03 (GF=0.1698 and GF=0.1651, respectively) followed by DRB1*13 (GF=0.1462). All genotype frequencies are in Hardy-Weinberg equilibrium. The most frequent haplotype in Sao Miguel is HLA-A*01-B*08-DRB1*03 (HF=0.0802) which is of West European origin. Albeit at lower frequency, we also found the Iberian North African haplotype HLA-A*30-B*18-DRB1*03 (HF=0.0047), the Iberian Berbers haplotype HLA-A*02-B*51-DRB1*13 (HF=0.0047) and two Mongol haplotypes: HLA-A*02-B*50-DRB1*07 (HF=0.0047) and HLA-A*02-B*44-DRB1*04 (HF=0.0142). Moreover, the preliminary interpopulation analysis highlights a strong relatedness with other European populations and input of people from different origins. These findings agree with our previous results on the Y chromosomal heritage of Sao Miguel population, and will be useful for Azorean studies of autoimmune diseases related to HLA genotypes. Funded by DRCT.(paularpacheco@hdes.pt).

FORENSIOMICS: Potential application of Ancestry Informative Markers to Forensics, Pharmacogenomics, and Outcomes Research. *M.F. Seldin¹, N. Yang¹, H. Li¹, L.A. Criswell², P.K. Gregersen³, M.E. Alarcon-Riquelme⁴, R. Kittles⁵, J.W. Belmont⁶.* 1) Univ Calif, Davis, CA; 2) Univ Calif, San Francisco, CA; 3) Univ Uppsala, Uppsala, Sweden; 4) North Shore Univ Hosp, Manhasset, NY; 5) Comp Cancer Cent, Ohio State Univ, Columbus, OH; 6) Baylor College of Medicine, Houston, TX.

We have identified or confirmed several hundred ancestry informative markers (AIMs) with large (>50%) allele frequency differences between different combinations of major ancestral groups including European(EA), West African(AF), East Asian(AS) and Amerindian(AI). A panel of >200 AIMs (optimized for ancestry information and distributed on each chromosome) was used to examine >750 DNA samples including those self identified as EA, AF, AS, AI, African American(AA), Mexican(MX), Mexican American(MA), Puerto Rican(PR) and South Asian(SAS). Analysis using the Bayesian clustering algorithm STRUCTURE 2.1 indicated that these data: 1) best fit a model in which individuals were derived from five major populations (k=5), and 2) showed grouping of individuals with similar ethnic identity without any identifier other than the AIMs genotyping information. In general, the 5 populations corresponded to the self identified ethnicities EA, AF, AS, AI and SAS (e.g.>97% EA had >97% probability of membership in population 1). As expected, other self identified groups showed admixture between the different populations and had distinct profiles (e.g. most MA clearly separated from PR). To further assess the potential of this approach, we used Fishers linear discriminate to examine the ethnic identity of each individual when treated as an unknown. For the majority of samples, the predicted ethnic identity corresponded with the self-identified identity at high probability (p>0.99). However, for some individuals alternative grouping was strongly suggested (e.g. 4 of 73 self described Amerindians were identified as MA at high probability). Overall, this study demonstrates the potential of developing a set of AIMs that can provide a useful adjunct to forensic medicine, pharmacogenomics and disease studies in which major ancestry may be linked to specific outcomes.

Departures from Hardy-Weinberg equilibrium across the human genome. *J.K. Wittke, C. Roe, N.J. Cox.*
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Hardy-Weinberg equilibrium (HWE) was first introduced over a century ago (Castle 1903; Hardy 1908; Weinberg 1908) and states that the allele frequencies (p and q , where $p+q=1$) determine the genotype frequencies at a polymorphic locus (e.g. p^2 , $2pq$, q^2), assuming the population is randomly mating and there is no mutation, selection, genetic drift, migration, population structure, or admixture. Departures from HWE in an unselected population sample can be due to violation of any of the underlying assumptions, genotyping error, or chance. We have examined genotypic data for departures from HWE over the human genome at 8,939 single-nucleotide polymorphisms (SNPs) in 150 unrelated Caucasians to characterize regions that show departures from HWE with respect to the extent and direction of departure. We used a χ^2 test with 1 degree of freedom, or Fishers exact test when the HWE-expected genotype counts were less than 5, to assess significance of departures from HWE. We measured linkage disequilibrium (LD) between SNPs across each chromosome using r^2 and D . The estimated genotyping error rate is 0.0004. For any level of significance chosen (0.05, 0.01, 0.001, etc.), we observe a higher proportion of SNPs showing departures from HWE at that significance level than expected by chance. Moreover, we observe a considerable excess of departures even when we create independent bins by clustering SNPs with $r^2 < 0.3$. Departures attributable to genotyping error or non-random patterns of missing data are unlikely to occur in each of several markers in strong LD, whereas departures due to chance or violations of HWE assumptions may be observed in several markers in strong LD. Any violation of the assumptions necessary for HWE must have occurred very recently in this populations history since departures from HWE remain in a population for only one generation, assuming the next generation is randomly mating and no other violation of the assumptions for HWE occurs. Further study is needed in other unrelated, Caucasian samples to better characterize which regions of the human genome show departures from HWE due to chance and which are violating HWE assumptions.

Historical and Genetic Assessment of A Genetic Isolate in North-West Colombia. *G. Bedoya¹, I. Soto-Calderon¹, J. Lopera¹, P. Montoya¹, V. Alvarez², A. Ruiz-Linares^{1,3}.* 1) Grupo de Genetica Molecular, Univ of Antioquia, Medellin, Colombia; 2) Dept. History, Univ of Antioquia, Medellin, Colombia; 3) Galton Laboratory, Univ College London, London, UK.

The Paisa population is a genetic isolate located in the province of Antioquia in North-west Colombia. In the oriental section of the province, the population of Marinilla and the Area of colonization (MAC) seem to have even higher levels of genetic isolation. This region exhibits high frequency of certain surnames and recessive autosomal traits but also had the highest fecundity rate in Colombia during the second half of the 19th century (6,7 children/family). In this research, current and ancient surname lists and records of marriages, as well as uniparental molecular markers of Y-chromosome (YC) and mtDNA were employed to determine the isolation level of MAC and to identify the parental populations.

The percentage of MAC with one of the 15 most frequent surnames was between 39 and 59%. The inbreeding coefficients due to random mating using surnames (ii) were between 0.0040 and 0.0135. The inbreeding calculated with marriages carried out between 1801 and 1850 was 0.0088 (closely equivalent to a union of third by second cousins, 0.0078). The haplotypic diversity of YC was 0.40 and the most predominant lineages were the Europeans (haplogroup A 71.4%, and C 16.9%). Based on STRs of YC, MAC had the shortest distance with Basques, Portugueses and Catalans. It was found a high ratio of Native American mitochondrial lineages A and B, which together account for the 77 to 100% of the localities of MAC. The mitochondrial haplotypic diversity was 0.57 and lower than most of Colombian populations excepting the Embera (Amerind).

These results indicate population growth with genetic isolation levels even higher than other human genetic isolates. Also, the molecular data show admixture in the foundation of MAC provided by females from Amerind populations isolated since Pre-Columbian times and Spaniard males principally of Basque origin. We thank to University of Antioquia (CPT0017) and Banco de la Republica (200212).

Correlation between constitutive skin pigmentation and individual ancestry in admixed populations. *A. Chan¹, M. Vaughn¹, R.A. Kittles², M.D. Shriver³, E.J. Parra¹*. 1) Department of Anthropology, University of Toronto, Mississauga, ON; 2) College of Medicine and Public Health, Ohio State University, Columbus, OH; 3) Department of Anthropology, Penn State University, University Park, PA.

We evaluated the correlation of constitutive pigmentation (melanin content in unexposed areas of the skin) and individual ancestry estimated with ancestry informative genetic markers (AIMs) in five admixed populations showing a wide range of constitutive pigmentation and admixture proportions (African Americans from Washington DC, African Caribbeans living in England, Puerto Ricans from New York, Mexicans from Guerrero and Hispanics from San Luis Valley). We also studied the relationship between pigmentation and individual ancestry by simulation, under several models differing in the presence or absence of admixture stratification (variation of ancestry between the individuals of the admixed sample), the presence or absence of pigmentation differences between the parental populations, and the number of genes determining melanin content. We observed a significant correlation between constitutive pigmentation levels and individual ancestry in each of the admixed samples. However, it is important to note that the strength of the relationship of constitutive pigmentation and ancestry estimated with genetic markers is quite variable. The correlations range from moderately strong (Puerto Rico, $\rho=0.633$) to weak (San Luis Valley, $\rho=0.259$; and Mexico, $\rho=0.212$). The variation observed in the extent of the correlation is presumably a reflection of differences in the degree of admixture stratification present in each population and/or the levels of pigmentation differences between the parental populations and the number of genes involved. Our results emphasize the need to be cautious when using pigmentation as a "marker" of ancestry, or when extrapolating the results observed in one admixed sample to samples from other admixed populations. Finally, we also discuss the implications of these results for population-based association studies and admixture mapping studies in admixed populations.

Performance assessment of methods for selecting haplotype tagging SNPs across four populations. B.V.

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The large amount of single nucleotide polymorphisms (SNPs) in the human genome creates a dilemma for geneticists: Do they genotype all available variants, possibly at an excessive cost and risking losing the true association in a sea of noise, or do they choose typing only a subset of the variants and hence possibly not genotyping the causal variation? Algorithms for selecting optimal haplotype tagging SNPs promise a means for dealing with this dilemma by selecting a subset of SNPs that represent all the haplotype variation in the complete set of SNPs. A conundrum of methods has been suggested for selecting tagging SNPs, which creates another dilemma for geneticists: which method should one use? These methods differ mostly in two major aspects: the correlation measure used to measure how well a set of SNPs captures the variance observed in the original dataset, and the algorithm used for the minimization of the final number of tagging SNPs. We evaluate a set of methods suggested based on three criteria: (1) the number of SNPs needed to capture variation in a study data set, (2) the extensibility of the set of tagging SNPs across different populations, and (3) the extensibility of the set of tagging SNPs to samples of the population of different sizes. Our results show that tagging SNPs that perform well in one population will generally perform well even in populations of different ancestry. In closely related populations, sets of tagging SNPs tend to be highly concordant. This reflects the ability of tagging SNP methods to capture common variation. Our results also show that methods considering only pair-wise correlation metrics are highly extensible across populations and sample size but give a larger set of tagging SNPs than multivariate metrics. We conclude that association studies attempting to find common causal variants in any population can be vastly simplified due to the background distribution of common variation, even if that background variation is ascertained in distantly related populations.

Direct determination of mutation characteristics of Y chromosome STR loci. L.A.D. Hutchison^{1,2}, N.M. Myres², J.E. Ekins², U.A. Perego², J.B. Ekins², K. Hadley², L. Layton², M.L. Lunt², S.S. Masek², A.A. Nelson², M.E. Nelson², K.L. Pennington², J.L. Peterson², A. Sims², T. Tolley², A. Welch², S.R. Woodward^{2,3}. 1) Department of Computer Science, Brigham Young University, Provo, UT, USA; 2) Sorenson Molecular Genealogy Foundation, Salt Lake City, UT, USA; 3) Department of Micro and Molecular Biology, Brigham Young University, Provo, UT, USA.

We present new analysis methods for haploid STR loci, which provide significant insight into per-locus mutation models and rates, as well as population substructure. These methods do not require knowledge of genealogical relationships between individuals, but are based on all-pairs comparisons between the individuals in a dataset (as employed by "mismatch distributions"). We formulate the *mutation model curve (MMC)* as the probability of matching at a specific locus, given that n out of N_L loci match in total between a pair of individuals; this function shows how quickly or slowly a locus mutates away from its original value, and the degree of back-mutation that occurs. We also present a method for quantification of population substructure in the data, and for calculation of per-locus mutation rates. The mutation model curve and the calculated mutation rate are given for each of 36 Y STR loci in a predominantly European dataset of 7976 individuals. The calculated mutation rates are shown to be comparable to rates obtained by observational studies. The mutation rates for many of the 36 loci in our study have not previously been determined by observation, yet mutation rates may be predicted for these loci by calibration to loci with known rates. These analysis techniques yield additional useful information about mutation characteristics of the loci, such as by inferring conditions under which identical-by-state matches are expected, and by demonstrating evidence in support of a stepwise mutation model.

A Nonparametric Approach for Population Structure Inference Using Multilocus Genotypes. *N. Liu*¹, *H. Zhao*^{1,2}.

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In genetic studies, characterizations of the population structure of species are very useful in a variety of contexts. Such as in evolution studies, much effort has been devoted for the estimation of the degree of population differentiation in order to investigate the evolutionary relationship of modern populations. In other situations, the presence of population structure poses a practical nuisance. Such as in genetic association studies using unrelated individuals, the presence of population stratification may lead to false-positive findings if it is not properly considered. We describe a two-stage approach in inferring population structure using multilocus genotype data. In the first stage we use dimension reduction methods such as singular value decomposition to reduce the dimension of the data (and noise), and in the second stage, we use clustering methods on the reduced data to identify population structures. The approach has the ability to identify population structure, make correct inference of the number of subpopulations, and assign individuals to its corresponding subpopulation. Our approach does not have any population genetics assumptions (such as Hardy-Weinberg equilibrium and complete linkage equilibrium between loci within populations) and can be used with any genotype data. We present results applying the approach to a mixture of two American populations. Compared to the most popular method (STRUCTURE, Pritchard et al. 2000; Falush et al. 2003), our approach has comparable performance and is faster and more efficient in inferring the number of clusters.

Genetic Structure of a Culturally Conserved Afro-Colombian Population. *I. Soto-Calderon¹, J. Lopera¹, MC. Mondragon¹, Y. Caicedo¹, L. Caraballo², G. Bedoya¹, A. Ruiz-Linares^{1,3}.* 1) Grupo de Genetica Molecular, Univ Antioquia, Medellin, Colombia; 2) Univ. de Cartagena, Cartagena, Colombia; 3) Galton Laboratory, Univ. College London, London, UK.

In 1774, the population of San Basilio de Palenque (PSB) was settled near Cartagena de Indias, the most important harbor of African slaves in the New World. This population kept in isolation until late 19th century, propelling the conservation of ancestral African costumes and the formation of a new tongue with Bantu influence.

Molecular data were used to determine the admixture level and to ascertain the predominant parental populations involved in the settlement of PSB. The markers used encompass 6 STRs and 4 biallelic loci in non-recombinant section of the Y-chromosome (YC), 6 biallelic loci in mtDNA, 4 autosomal loci with population specific alleles (PSA) and 6 sites located in the α -globin gene cluster.

It was found 83% of African (haplogroup E) or potentially African YC haplotypes (C, D) in PSB, which is consistent with high levels of African ancestry identified with the PSA (90%). Other YC haplotypes found in PSB indicate 4% of Amerindian lineages (haplogroup B) and 13% European. The average gene diversity calculated with 6 STRs in YC was 0.50.

In mtDNA, it was found 69% of lineages proceeding from Sub-Saharan Africa (haplogroup L), 8.4% Amerindian (A-D) and 22.6% non-Amerindian, whose origin remains to be established. Also, the haplotypic diversity (0.58) was lower than that reported for other admixed and Amerind Colombian populations.

The allelic frequency of the ^s mutation (E6V) in the gene was 8.8%. 84% of the haplotypes linked to E6V in PSB have been previously reported in African populations exhibiting 52% of Benin, 28% of Cameroon and 4% of one haplotype found in Central African Republic and Senegal. As the origin of the E6V mutation has been traced to Africa and regarding the high degree of African ancestry in PSB, it can be suggested recombination between 5' and the gene .
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Genetic contribution of Acadian founders to the contemporary Quebec population. *M. Tremblay, J. Bergeron, L. Houde, H. Vézina.* Univ Quebec, Chicoutimi, PQ, Canada.

The year 2004 marks the 400th anniversary of the arrival of the first French settlers in the Acadian peninsula. The present Acadian population of North America is descended from these early 17th century French immigrants who settled in an area located in the province of Nova Scotia (Eastern Canada). In 1713, this French territory was yielded to the British Crown through the signature of the Utrecht Treaty. Forty-two years later, the British authorities ordered the deportation of the Acadians who were dispersed in the British colonies of America. Some of the deportees settled in Canada, which became a British colony in 1763 (Treaty of Paris). It is estimated that between 2000 and 3000 Acadians have settled in the territory of what is now the province of Quebec, during the second half of the 18th century. In Quebec, some parishes were founded and populated almost exclusively by Acadians, while others received a considerable number of Acadian migrants. The goal of this study is to evaluate and characterize the impact of the Acadian migratory input on the Quebec gene pool by measuring and comparing its demographic and genetic contributions to the various regions of Quebec. Analyses are based on genealogical data contained in a corpus of 2340 ascending genealogies (BALSAC-RETRO database). These genealogies cover the entire Quebec territory and go back to the beginning of the 17th century, with a maximum lineage depth of 16 generations. Acadian founders were identified, and their period of marriage, frequency of occurrences in the genealogies and genetic contributions to the regional populations of Quebec were analyzed. Although Acadian founders can be found in most genealogies, results point toward an important variability of the Acadian imprint on Quebec's regional gene pools.

Genetic analysis of caste and tribal populations of south India. *W.S. Watkins¹, B. Mowry², T.N. Srinivasan³, R. Thara³, B.V.R. Prasad⁴, J.M. Naidu⁴, B.B. Rao⁴, M.J. Bamshad^{5,1}, L.B. Jorde¹.* 1) Department of Human Genetics, University of Utah, Salt Lake City, UT 84112; 2) Queensland Centre for Mental Health Research, Wacol, Brisbane, Australia 4076; 3) Schizophrenia Research Foundation India, Chennai, Tamil Nadu, India; 4) Department of Anthropology, Andhra University, Visakhapatam, India; 5) Department of Pediatrics, University of Utah, Salt Lake City, UT 84112.

The diverse populations of the Indian subcontinent provide an opportunity to examine human evolution and population affinities influenced by well-defined social customs. To test hypotheses of population differentiation, we genotyped 43 unlinked short tandem repeats (STRs) in 179 individuals from 4 castes of varying social rank (2 Brahmin (upper), Mudaliar (middle), Harijan (lower)) from the state of Tamil Nadu and 152 individuals from 8 tribal groups (Chenchu, Irula, Kattunaicken, Khonda Dora, Maria Gond, Santal, Siddi, Yanadi) from south India. STR heterozygosity is similar between caste groups (mean = 0.683, range 0.665 - 0.697). In contrast, tribal populations vary substantially for STR heterozygosity (mean = 0.665, range 0.731 - 0.538), suggesting that substantial genetic drift has occurred in these isolated populations. R_{ST} estimates are low for Tamil castes (0.007), Andhra castes (0.010), and between Tamil and Andhra groups (0.002). The R_{ST} estimate for tribal populations (0.042, Siddi excluded) is more than 4 times greater than that for all south Indian castes (0.010) and, interestingly, greater than the value for 7 sub-Saharan African populations ($R_{ST} = 0.026$). For the 3 Tamil speaking castes, genetic distance estimates (dsw method) followed caste status (Brahmin/Mudaliar = 0.0011, Brahmin/Harijan = 0.0021, Mudaliar/Harijan = 0.0015) showing a positive, although non-significant, correlation ($r = 0.92$, $p > 0.32$). Caste populations from Andhra Pradesh show a similar pattern. The trend was not observed when the non-Tamil speaking Brahmin group was included, possibly reflecting the effects of language on mating patterns.

The use of Short Tandem Repeats Loci to study the genetic structure of several populations from Zulia State, Venezuela. *W. Zabala¹, L. Pineda¹, L. Borjas¹, E. Fernández¹, MG. Portillo¹, MA. Sanchez¹, A. Aranguren², W. Delgado¹, JA. Chacín¹.* 1) Medical Genetic Unit, Univ Zulia, Maracaibo, Venezuela; 2) Department of Genetics, Faculty of Veterinary, Univ Zulia, Maracaibo, Venezuela.

Short Tandem Repeats (STR), is a group of highly polymorphic markers used mainly as markers of genetic individuality in criminalistic and paternity testing. However, the distribution of allelic frequencies has shown significant differences when comparing them between ethnic groups, suggesting that these systems can be used to detect genetic differences between populations and sub-populations. The aim of this study is to evaluate the utility of autosomal STR loci to define the genetic structure of five populations in Zulia. The sample consisted of unrelated individuals from Maracaibo, Isla de Toas, San Jose de Heras (SJH), Bari and Yukpa. DNA was extracted and seven autosomal STR loci were amplified (CSF1PO, TPOX, TH01, VWA, D7S820, D13S317 and D5S818). PCR products were characterized by PAGE 5%, and stained with silver nitrate. The allelic frequencies, HW-Eq, Obs and Expect Heterozygosity, the Wrights F-statistic, were determined. It was constructed a pair-wise distance matrix between populations using the coefficient of gene differentiation or F_{ST} . A dendogram with the algorithm UPGMA using the Nei's D_A distance was used to compare the zulian populations with others in the world, and also the principal components analysis was used to depict the genetic distances in three dimensions. It may be observed that there are differences in the distribution of the allelic frequencies for each population, allowing identification of their own characteristics, probably related to the origin of each one. The major genetic diversity was found in SJH. It was also proved the existence of a general tendency of not random crosses that causes a departure from panmixia in the studied population. The dendogram clustered the zulian populations with those groups that have greater affinity. These results are supported with the estimation of the percentage of ethnic admixture and the historic evolution of the region emphasizing the importance of considering history for a suitable interpretation of genetics data.

The DRD2 A1 allele Contributes to the Severity of opium-dependence in the Iranian Population. M.

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Dopaminergic mechanisms are thought to play an important role in the pathogenesis of certain neuropsychiatric disorders including drug abuse. Little attention has been focused on the molecular genetic factors that might influence the prognosis and severity of opiate-dependence. We studied the allelic association between DRD2 Taq A1 polymorphism and daily drug usage in 100 opium-dependent Iranian patients and 130 unrelated controls. A 310 bp region surrounding Taq I site at the DRD2 locus was amplified by polymerase chain reaction (PCR) and the PCR product was digested with Taq I restriction enzyme. The A1 allele remained intact while the A2 allele was cut. Significant association was observed between the A1 allele and the average use of opium (g/day) in opium-dependent patients; the A1A1 genotype correlated with higher daily usage comparing with the A1A2 or A2A2 genotypes ($P=0.0001$). Our results support a role for DRD2 in the severity of opium addiction in the Iranian population.

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Intronic Regions Involved in Splicing Show Less Polymorphism. *B. A. Salisbury, C. Harris-Kerr, J. C. Stephens.*
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Variation found in introns may have phenotypic relevance either through regulatory or splicing effects. We analyzed the distribution of intronic SNPs discovered in the course of resequencing over 5000 genes. We discovered a clear pattern of greatly reduced variability in the splice donor and acceptor regions of introns. The detailed patterns we observed correspond well with what is known about the recognition of splicing elements by components of the spliceosome. Furthermore, the SNP pattern is consistent with the patterns reported by others of nucleotide variation among introns; i.e., sites that have a limited nucleotide variability among introns have limited polymorphism within introns. We observed about 90% fewer SNPs in the two 5'-most and two 3'-most nucleotide positions relative to the most SNP-dense positions. A stretch of significantly reduced SNP density was also observed in the poly-pyrimidine tract near the acceptor splice site. Certain other positions also showed significant differences in SNP density. Finally, differences in SNP density correlate strongly with differences in allele frequencies for the SNPs that exist; at positions with fewer SNPs, the SNPs that are found tend to have low minor allele frequencies. We have additionally considered the spectrum of nucleotide changes reflecting allele status as common or rare and ancestral or derived relative to chimpanzee sequence.

L1-mediated mobilization of trans-spliced and chimeric transcripts: a mechanism to increase genomic diversity.

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LINEs are thought to have contributed to more than 40% of the human genome through direct insertion, by trans-mobilization of Alus, SVAs, and processed pseudogenes, and by providing substrates for chromosomal rearrangements. L1s have the potential for exon-shuffling by carrying along 5'-transduced sequences when transcribed from a fortuitously-located upstream promoter, or 3'-transduced sequences by forgoing their own polyadenylation signal for a stronger downstream one. Here we report a novel mechanism of L1-mediated exon-shufflingretrotransposition of trans-spliced RNAs. We previously described an active L1 tagged with a bi-directional splice acceptor gene-trap. We now demonstrate precise splicing of the gene-trap cassette to the upstream splice donor when integrated into an intron. Using TAIL-PCR we have characterized genomic locations of 36 insertions. One insertion landed into intron 1 of the PKIA gene on 8q21 and had all the hallmarks of a bone fide retrotransposition event. Unexpectedly, the inserted sequence was a chimera formed by two trans-spliced RNAs the 3'-most 119 bp of exon 1 of the heterologous transcript FLJ23285 was spliced precisely to the 5' splice acceptor of the gene-trap cassette. Using RT-PCR we ruled out the occurrence of a prior insertion into FLJ23285, followed by a 5'-transduction event, splicing and integration of the transcript into 8q21. To assess the extent of such events, we examined 10,000 human processed pseudogenes for evidence of retrotransposed fused exonic sequences. One such chimera was detected on chr. 17, in which 5 exons of HMG-4 gene from chr. X were fused 3' to 3 exons of ATP synthase gene on chr.11, all flanked by TSDs characteristic of L1-mediated retrotransposition. The fusion site was most consistent with this being a product of template switching during the TPRT reaction. Thus, we demonstrate the potential of L1s to mobilize trans-spliced and otherwise fused transcripts, contributing to exon and domain shuffling and increasing genomic diversity.

Unexpectedly high allelic variation found in a locus of young and active L1 elements. *C.N. Talchai, M.dC. Seleme, H.H. Kazazian.* Dept. of Genetics, School of Medicine, University of Pennsylvania, Philadelphia, PA.

L1 retrotransposons are the only family of active transposable elements in the human genome as indicated by multiple cases of *de novo* insertions, cell culture assays, retrotransposition in transgenic mice and database analyses. The overall L1 family comprises about 17% of our genome, but only a small proportion of elements (<0.2%) retain open reading frames and are potentially active. Among these intact L1 elements, 38 of 86 (44%) are polymorphic as to their presence or absence in human populations. The isolation of the first active element (L1.2) revealed that L1 elements present alleles just as regular cellular genes do. Recently it was shown that two alleles (L1.2A and L1.2B) of this element display a 16-fold difference in retrotransposition capacity, suggesting that allelic heterogeneity can influence the potential mutational load of an individual genome. Here, we further investigated the allelic status of a highly active and polymorphic L1 (A1512428). We isolated the full-length L1s from heterozygous individuals of diverse ethnic origins and compared their sequences. By direct sequencing, we verified that no PCR errors were introduced during the 6kb amplification. The copies of the element tested from five different individuals were very variable, with two alleles almost identical to the database reference sequence and two others identical to each other, but with 6 nucleotide changes from the reference. Some changes were shared among different alleles. Intriguingly, the fifth allele contained 9 changes from the reference element: among the 4 private ones, 2 introduced stop codons into ORF1. This is the first example of allelic differences in a young L1 locus that features both mutated (likely dead) and active alleles, adding an important level of complexity to our understanding of individual genome dynamics. We hypothesize that other polymorphic L1 loci contain such allelic variability, and are further investigating allelic variation and retrotransposition activity of these polymorphic L1s.

Prevalence of the FMR1 mutation in Taiwan assessed by large-scale screening of newborn boys and analysis of DXS548-FRAXAC1 haplotype. *C.C. Tzeng¹, L.P. Tsai², W.L. Hwu³, S.J. Lin⁴, M.C. Chao⁵, Y.J. Jong⁵, S.Y. Chu⁶, W.C. Chao¹, C.L. Lu⁷.* 1) Department of Pathology, Chi Mei Medical Center, Tainan; 2) Department of Pediatrics, Taipei Municipal Womens and Childrens Hospital, Taipei; 3) Department of Pediatrics and Medical Genetics, National Taiwan University Hospital, Taipei; 4) Bureau of Health Promotion, Department of Health, Taipei; 5) Departments of Pediatrics and Medical Genetics, Kaohsiung Medical University, Kaohsiung; 6) Departments of Pediatrics, Buddhist Tzu Chi Hospital, Hualien; 7) Department of Medical Research, Chi Mei Medical Center, Tainan, Taiwan.

If carrier women could be identified in time and take appropriate measures, fragile X syndrome can be prevented. Wide screening of women to be or in their early pregnancy was considered a good approach to identify carriers without misdetection. Nevertheless, we argued against the cost-effectiveness of implementing such a screening program in Taiwan, due to the lower carrier rate found in our pilot study. To reliably estimate the prevalence of mutant FMR1 gene in Taiwan, we anonymously screened 10,046 newborn boys using bloodspot PCR. Among them, 5 had Z (61-200 CGG repeats) or L (full mutation) allele, revealing a prevalence of one per 2,009 X chromosomes, which is significantly lower than a prevalence of one per 462-482 X chromosomes in Caucasians (p 0.05). The prevalence of S (41-60 CGG repeats) allele was one per 50 X chromosomes (201/10,046), also significantly lower than one per 33-35 X chromosomes in Caucasians (p 0.05). Furthermore, when comparing analyses of the distribution of alleles at the two most often investigated microsatellite loci, DXS548 and FRAXAC1, between 100 control and 28 unrelated fragile X chromosomes, we found no apparent founder haplotype prevalent among the fragile X patients. Because a few founder haplotypes were reportedly prevalent in two thirds of fragile X alleles in Caucasians and Chinese in central China, we thus suggested that lack of founder fragile X chromosomes might result in a relatively low prevalence of mutant FMR1 gene in a population, as observed in Taiwan.

Comparative analysis of human and chimpanzee duplicated regions, located in an unstable loci in distal Xq28. *R. Bandyopadhyay, D.L. Nelson.* Baylor College of Medicine, Department of Molecular and Human Genetics, Houston, TX.

Analysis of the human genome sequence shows that 5% of the human chromosome is composed of intra and inter chromosomal duplications. Segmental duplications play significant roles in human disease. Segmental duplications on sex chromosomes constitute an important proportion of recent duplications. Incontinentia Pigmenti (IP) is a X-linked, male lethal disorder characterized by abnormal skin pigmentation, retinal detachment, alopecia, and mental retardation. The majority of the IP cases have an identical deletion in the NEMO gene that eliminates exons 4 to 10. The gene is located on Xq28, immediately distal to G6PD. A 35.5kb region containing the majority of the NEMO gene is duplicated, and the inverted copy found about 22 kb distal is more than 99% identical in sequence. Comparative studies in closely related species is one approach to understand the origin and maintenance of these duplicated sequences; this duplication is found in both chimpanzee and gorilla. We analyzed the sequence divergence of the orthologous chimpanzee locus (NEMO-duplications) for both the flanking and the duplicated regions. The result showed reduced divergence of the duplicated sequence compared to the flanking sequence. The overall nucleotide substitution level in aligned regions between PTR-NEMO dup and HSA-NEMO dup is about 1% (excluding indels), which is significantly lower than the reported estimates of divergence, ranging from 1.23% to 1.44%. We identified 38 indels in total. The overall divergence between the two sequences is ~ 2%, counting the indels, which is again lower compared to 5%, when indels are counted. The longest indel identified in this region is 91bp and it is within an Alu sequence in the noncoding region. Other segmental duplicated regions present in Xq28 were also analyzed. All these regions show high sequence conservation between the PTR and HSA. The high sequence similarity between the duplicated regions may be due to frequent gene conversion and possibly a directional bias in gene conversion is responsible for the lack of drift within each sequence.

A New Technique for the Rapid and Inexpensive Genotyping of Y Haplogroups. *L.R. Harrell¹, L. Zhou², L. Jorde¹.*
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We present the application of a new method for performing inexpensive and rapid genotyping of the Y chromosome. This method amalgamates a few straightforward techniques into a formidable genotyping system. The first step is the asymmetric PCR of the region immediately surrounding the SNP (or small insertion/deletion) which generates an excess of one of the template strands. The PCR product is then combined with a dye that binds double-stranded DNA (LC Green I) and a 3-blocked oligonucleotide whose sequence straddles the SNP, mimicking the template strand that was depleted in the PCR reaction. The mixture is then completely denatured, cooled, and slowly reheated on either a LightCycler (for a single sample) or a LightTyper (for 96- or 384-well plates of samples), generating a melting curve for the duplex. Due to the nature of the oligo, two scenarios are possible; either the oligo will be a perfect match to the SNP or it will contain a 1bp mismatch because the oligo doesn't match template at the location of the SNP. The difference between these two possibilities shifts the melting curve of the duplex sufficiently to be diagnostic of the allelic state of the SNP. Additional benefits, such as the lack of a need to eliminate mispriming events and our ability to multiplex the PCR reactions, make this method both efficient and cost effective. We have used this new method to design a genotyping panel for the Y chromosome that is able to assign individuals into 1 of 38 haplogroups (A1 through R2) based on the Y Chromosome Consortium tree. We have used this panel to genotype nearly 1000 individuals with a special focus on populations from southern India. Population substructure and migration histories were examined and compared to previous findings for Y chromosome and mtDNA HVS I sequences.

What Can Environmental Genome Project Data Tell Us About Gene Haplotype Structure? *R.W. Morris¹, P.D. Terry², L. Li³, N.L. Kaplan³, J.A. Taylor^{2,4}*. 1) Dept of Anesthesiology, Duke Univ Medical Ctr, Durham, NC; 2) Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, NC; 3) Biostatistics Branch, National Institute of Environmental Health Sciences, Research Triangle Park, NC; 4) Laboratory of Molecular Carcinogenesis, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

The Environmental Genome Project (EGP) is a publicly available data set of resequenced DNA repair and cell cycle control genes in a sample of 90 people of diverse ethnicity. We characterized genotype structure in 106 genes using 2058 common SNPs (minor allele sample frequency > 0.1). 381 different observed ensembles of minor alleles were directly observed in individuals homozygous for either the major or minor allele at each SNP. Forty additional inferred ensembles of SNPs were identified where pair-wise genotype correlation coefficients exceeded 0.80. On average, there were 19 common SNPs representing 4 ensembles per gene. These ensembles often consist of unique sets of minor alleles and are thus "orthogonal" to one another. Observed and inferred ensembles together can be used to explain 97% of the SNP genotype variation in the EGP sample. How well can these ensembles explain SNP genotype variation in other data? To answer this question, we used data from the HapMap project. For SNPs present in both data sets, the ensembles from the EGP data matched haplotypes inferred from HapMap genotypes of CEPH families. Many of the ensembles from the EGP could not be evaluated because of the paucity of HapMap SNPs within the relevant genes. In addition, although the ethnicity data for the EGP data has been destroyed, we have used Cancer500 data to confirm that common EGP SNPs are multi-ethnic. We conclude from these comparisons that SNP ensembles found in the EGP data accurately represent haplotype variation found in HapMap CEPH families, but that, because of low SNP density, HapMap currently fails to sample many additional common gene haplotypes that can be found using resequencing data. In addition, common SNPs from EGP are pan-ethnic, suggesting that the resulting haplotypes are also pan-ethnic.

Hearing impairment associated with DFNA5 is caused by an unusual mechanism where skipping of one specific exon leads to disease, that is not caused by other mutations in this gene. *G. Van Camp¹, K. Vrijens¹, M. Pfister², S. Thys¹, M. Mueller², V. Van Tendeloo³, L. Umans⁴, L. Serneels⁴, F. Kooy¹, R. Smith⁵, D. Van Bockstaele³, F. Van Leuven³, J.-P. Timmermans⁶, L. Van Laer¹.* 1) Dept. of Medical Genetics, University of Antwerp, Belgium; 2) Hals-Nasen-Ohrenklinik, University of Tübingen, Germany; 3) Laboratory of Experimental Haematology, University of Antwerp, Belgium; 4) Experimental Genetics Group, University of Leuven, Belgium; 5) Molecular Otolaryngology Research Laboratories, University of Iowa; 6) Laboratory of Cell Biology and Histology, University of Antwerp, Belgium.

Three mutations have been described in the DFNA5 gene in 3 families with autosomal dominant non-syndromic hearing impairment. Although different at the genomic DNA level, these mutations all lead to skipping of exon 8 at mRNA level. DFNA5^{-/-} mice were generated through deletion of exon 8 by targeted recombination, simultaneously mimicking the human mutation. To test the hearing, frequency-specific ABR (Auditory-evoked Brainstem Response) measurements were performed. Transfection experiments in mammalian cell lines (HEK293T; COS-1) using GFP-tagged wild-type and mutant DFNA5 were performed and cell death was analysed using flow cytometry and fluorescence microscopy. ABR tests could not demonstrate significant differences between DFNA5^{-/-} mice and their wild-type littermates. After transfection with mutant DFNA5-GFP, cell death approximately doubled when compared with transfection with wild-type DFNA5-GFP. The fact that DFNA5^{-/-} mice have normal hearing, in combination with the fact that only mutations that lead to exon 8 skipping have been described for human hearing loss, led to the hypothesis that hearing impairment associated with DFNA5 is caused by a highly unusual mechanism where skipping of one specific exon leads to disease, that is not caused by other mutations in this gene. We hypothesize that this represents a gain-of-function mutation, with the truncated protein exerting a deleterious new function. This hypothesis was supported by the fact that transfection with mutant DFNA5-GFP significantly increased cell death.

Spectrum and functional analysis of inactivating mutations in the epidermis-type lipoxygenase genes in autosomal recessive congenital ichthyosis (ARCI). *K.M. Eckl¹, P. Krieg², W. Küster³, H. Traupe⁴, F. André¹, N. Wittstruck¹, G. Fürstenberger², H.C. Hennies¹.* 1) Molecular Genetics and Gene Mapping Center, Max Delbrück Center, Berlin, Germany; 2) Eicosanoids and Tumor Development, German Cancer Research Center, Heidelberg, Germany; 3) Tomesa Fachklinik, Bad Salzschlirf, Germany; 4) Dermatology, University of Münster, Germany.

Autosomal recessive congenital ichthyosis (ARCI) forms a clinically and genetically heterogeneous group of severe inherited keratinization disorders characterized by intense scaling of the skin, different in color and shape, and often associated with erythema. Mutations in *ALOX12B* and *ALOXE3* on chromosome 17p13, coding for epidermal lipoxygenases 12R-LOX and eLOX-3 preferentially synthesized in the skin, were previously found in patients with ichthyosiform erythroderma. 12R-LOX and eLOX-3 are subsequent members of the same pathway, which converts arachidonic acid to an epoxyalcohol. In a large group of 150 families with ARCI, we identified 20 novel and one previously described point mutations in *ALOXE3* and *ALOX12B* in 20 families from various ethnic backgrounds. We found only few mutations repeatedly in different families but no founder effects as demonstrated by microsatellite analysis. We further analyzed the expression of the mutant genes and developed an in vitro assay in order to determine the enzyme activity using the genuine substrates. Analysis of reaction products by reversed-phase HPLC demonstrated that all missense mutations but one depleted the enzymatic activity of recombinant enzymes. Moreover, we intend to study subsequent changes in the expression of further interacting proteins of keratinocytes. All patients were clinically characterized in detail. We observed that mutations in epidermal lipoxygenase genes resulted in a mild phenotype of ARCI with light scales, normal hair, mild or absent palmoplantar hyperkeratosis, and hypohidrosis. We did not see dark brown scales on the face and/or adherent scales on the trunk. This is the first time that genetic findings in patients with ARCI could be correlated with the phenotype, whereas mutations in the gene *TGM1* were seen with the whole spectrum of mild to very severe forms of ARCI.

Single-stranded DNA fragments isolated from plasma of healthy subjects. *J.J. Jonsson^{1,2}, A. Alfredsson², J. Johannsdottir^{1,2}, G.H. Gunnarsson^{2,3}, M. Konradsson^{1,2}, S. Kristjansdottir², J. Asmussen².* 1) Dept Genetics/Molecular Med, Landspítali-Univ Hosp, Reykjavik, Iceland; 2) Dept. of Biochemistry and Molecular Biology, Faculty of Medicine, Univ. of Iceland; 3) BioCule, Reykjavik, Iceland.

Aims: DNA is present in low concentration in plasma. Limited information is available on its characteristics and function in health and disease. We developed four assays for plasma DNA concentration and structure and analysed samples from healthy adult subjects. **Methods:** DNA was isolated from plasma samples collected from 20 healthy individuals (21-33 yrs.) using High Pure Viral Acid Kit (Roche Diagnostics). DNA concentration was measured with an ultrasensitive LightCycler qPCR assay amplifying an 169 bp segment from Alu repeats with real-time SYBRGreen I fluorescence and a direct PicoGreen fluorometric assay for dsDNA. Size of dsDNA fragments was determined with PAGE and size and fraction of dsDNA and ssDNA fragments with 2D strandness-dependent electrophoresis (2D-SDE) and fluoroimaging. **Results:** We developed two quantitative methods based on different analytical principles since no standard for plasma DNA is available. Average concentration of plasma DNA was 4,7 g/L (range 1,77,8) according to the qPCR-Alu method and 3,1 g/L (range 1,06,0) with the direct dsDNA fluorometric assay. Correlation coefficient between the two methods was only 0.7. dsDNA in plasma DNA of healthy individuals was between 150 to 300 bp in length on electrophoresis. 2D-SDE revealed that the majority of plasma DNA was single stranded, explaining the bias and relatively poor correlation between the two quantitative methods. **Conclusions:** We have developed four sensitive assays to characterize plasma DNA in normals and various disease states. Surprisingly, the majority of plasma DNA isolated from normals was single stranded and corresponded to 1-2 nucleosomes in length. In contrast, current models of apoptotic and necrotic cellular release of DNA favor double-stranded fragments. Future studies should address to what extend strandness of plasma DNA fragments is dependent on methods of isolation and poorly characterized biological factors.

Characterization of human homologs of yeast ubiquitin-conjugating enzyme Ubc6p: Phosphorylation of hsUbc6e. *R.S. Oh^{1,2}, X. Bai¹, J.M. Rommens^{1,2}*. 1) Genet & Genom Biol, The Hospital for Sick Children, Toronto, ON, Canada; 2) Mol & Med Genet, U of Toronto, Toronto, ON, Canada.

Mutant membrane proteins that are associated with human disease are frequently targeted for degradation from the endoplasmic reticulum (ER). Nascent terminally misfolded or misassembled membrane proteins are subject to retrotranslocation from the lumen of the ER to the cytosol where ubiquitination machinery localized at the cytosolic face of the ER targets them for the proteasome. Ubiquitin-conjugating enzyme Ubc6p, a tail-anchored protein which is localized to the cytoplasmic face of the ER membrane, has been implicated in the degradation of several misfolded membrane proteins in yeast including mutant ABC transporters Ste6p and Pdr5*, and also heterologously expressed CFTR. We have undertaken characterization studies of two human homologs of Ubc6p, hsUbc6 and hsUbc6e. Both display high conservation to the yeast protein in their catalytic domain and possess a tail-anchored membrane protein motif. Both are functional ubiquitin-conjugating enzymes as determined by in vitro thiol-ester assay. Both also displayed induction by unfolded protein response, a feature of many ER-associated degradation (ERAD) components. Post-translational modification of hsUbc6e was observed and identified as phosphorylation at Ser184, which resides within the uncharacterized region linking the highly conserved catalytic core and the carboxyl terminal transmembrane domain. The modification was evident soon after translation, was stable over time in pulse-chase metabolic labeling experiments, and required membrane localization. Assays of hsUbc6e mutants S184D and S184E, which mimic the phosphorylated state, suggest that phosphorylation may reduce capacity for formation of ubiquitin-enzyme thiol esters. The occurrence of two distinct Ubc6p homologs in vertebrates, including one with phosphorylation modification, emphasizes diversity in function via modulation of thiol ester formation and possibly substrate recognition in ERAD processes. Further studies will be aimed at elucidating these distinctions as well as involvement of these human homologs in the degradation of wild-type and mutant CFTR.

Combined bioinformatic and biochemical analysis discriminates between neutral and clinically significant genetic variation in ABCA1. *L.R. Brunham¹, R.R. Singaraja¹, P.D. Thomas², M.R. Hayden¹.* 1) Dept Medical Genetics, CMMT, Univ British Columbia, Vancouver, BC, Canada; 2) Applied Biosystems, San Francisco, CA, USA.

ABCA1 is the key regulator of cellular cholesterol efflux. Many mutations and SNPs have been described in the ABCA1 gene, which result in a broad spectrum of clinical phenotypes of HDL-C levels and susceptibility to atherosclerosis. A major challenge remains the assignment of function to these genetic changes. We used data from PANTHER, a bioinformatics tool, to predict the functional consequences of each SNP and mutation in ABCA1, and validated these predictions through biochemical assays of function. PANTHER consists of a collection of protein families and subfamilies that allows us to ask the question, how likely is a given amino acid to occur at a given position in related proteins from the same and other organisms? By comparing the probabilities of the wildtype amino acid and its substitution, we can calculate a score to predict the functional consequence of a given substitution. Compared to mutations (n=15), SNPs (n=9) score much closer to zero (mean -1.57 1.04 vs. -5.32 1.68, Mann-Whitney $P < 0.0001$), indicating that SNPs are predicted to result in much milder effects on ABCA1 function compared to mutations. The two mutations with the most neutral subPSEC scores, D1289N and P2150L, both scored in the range observed for most SNPs. We tested the ability of proteins harbouring these mutations to elicit cholesterol efflux and found no significant change relative to wildtype ABCA1, suggesting that these mutations are in fact functionally neutral. Conversely, the mutations with the most negative subPSEC scores (N935H, N935S and Q597R) uniformly result in severe phenotypes in vivo. We tested the ability of two of these mutations to elicit lipid efflux. Relative to wildtype, these mutations resulted in significantly reduced cholesterol efflux (N935S: 29.31% 13.0; Q597R: 17.73% 13.77), underlying the severe clinical phenotype observed in patients with these mutations. These results validate the use of PANTHER in making predictions of the functional consequences of genetic variation.

Bioinformatic identification of regulatory regions within *RBI* introns. *S. Lithwick*^{1,2}, *V.D. Brown*², *W.W. Wasserman*³, *T.W.Y. Wang*², *B.L. Gallie*^{1,2}. 1) Department of Molecular & Medical Genetics, University of Toronto, Toronto, Ontario, Canada; 2) Department of Cancer Informatics, Princess Margaret Hospital, Toronto, Ontario, Canada; 3) Centre for Molecular Medicine & Therapeutics, Vancouver, British Columbia, Canada.

Retinoblastoma is a rare childhood retinal cancer, resulting from mutations within the *RBI* tumor suppressor gene. Coding mutations are implicated in 89%, and promoter mutations in 5% of probands. No *RBI* mutation has been identified in 6% of probands. Regulatory domains additional to the promoter have been identified within introns using cross-species comparisons; functionally significant sequences are maintained while surrounding sequences diverge. We hypothesize that some of the probands in whom no *RBI* mutation is yet identified have mutations within non-coding regions that regulate *RBI* gene expression. We used phylogenetic footprinting to identify *RBI* promoter and intronic regions that are conserved between species. Conserved regions were analyzed using a suite of in-silico motif prediction and sequence analysis programs. Bioinformatic predictions were evaluated using electromobility shift assays (EMSA), chromatin immunoprecipitation (ChIP), and luciferase transcription assays. Transcription factor binding sites (TFBSs) were predicted within the *RBI* core promoter and a 5' portion of intron 1 conserved between human, mouse, and rat. All functionally implicated sequences were independent of simple genomic repeats, and did not resemble either snoRNAs or microRNAs. All of the conserved sequences were biased for GC basepairs. EMSA assays suggested that proteins interact with each of the conserved regions, and ChIPs indicated that one protein interactor may be E2F. Non-coding sequence regions conserved among *RBI* orthologs may play an important role in the regulation of gene expression at discrete stages of mammalian development. These conserved regions are candidate sequences in which to search for mutations that predispose to retinoblastoma.

SCA1 pathogenesis: An alteration in ROR mediated gene expression. *H. Serra*¹, *J. Lande*^{1,3}, *H.Y. Zoghbi*², *H.T. Orr*¹. 1) Lab Medicine & Pathology, Institute of Human Genetics, Univ Minnesota, Minneapolis, MN; 2) Howard Hughes Medical Institute, Dept Molecular and Human Genetics, Baylor College of Medicine, Houston TX; 3) Dept of Medicine, Univ Minnesota, Minneapolis, MN.

Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant neurodegenerative disease caused by the expansion of a polyglutamine repeat within the disease protein, ataxin-1. We used microarray analysis to better understand the pathophysiology of SCA1. Initially we identified a set of downregulated genes related to glutamate signaling in the cerebellum. With further analyses we detected a decrease in the retinoid-related orphan receptor-mediated (*ROR*) gene expression in the line carrying mutant ataxin-1 when compared to the non-ataxic line. In the mutant line a decreased expression of genes related to synaptic calcium signaling as well as to glutamate signaling were observed. Most of these genes are known to have their expression controlled by ROR. To examine the relationship between ROR and ataxin-1 we performed coimmunoprecipitations using cerebellar extracts from a wild type mouse to demonstrate that ROR and ataxin-1 interact in vivo. The interaction between ROR and ataxin-1 is under further investigation. Together with other data these results point to a direction where ataxin-1 participates in a nuclear complex involving gene transcription and RNA regulation.

A systematic approach for functional annotation of human KAO-NASHI (Face-less) genes. *N. Shimizu¹, A. Shimizu¹, T. Sasaki¹, M. Furutani-Seiki², H. Kondoh², S. Asakawa¹*. 1) Dept Molecular Biol, Keio Univ Sch Medicine, Tokyo, Japan; 2) Kondoh Research Group, SORST, JST, Japan.

The Human Genome Project has provided the DNA sequences of 3 billion base pairs and identified over 30,000 protein-coding genes in the human genome. However, many of these protein-coding genes are not fully proven by experimental evidences. In general, proteins with known motifs are readily classified, but substantial numbers of protein have no obvious motifs in their sequences. We designated these genes/proteins without obvious motifs as KAO-NASHI (Face-less) and initiated a project to unveil their face (kao) by comparative genomics and knockdown analysis using Medaka (a small teleost fish) as animal model.

First, we made a list of human genes/proteins, and analyzed whether they have known motifs using HMMER program and Pfam database or trivial or nave names of obvious function. All genes/proteins were filtered by motif names or gene names and about 4,000 genes remained after this filtration. Genomic structures and open reading frames were carefully inspected to remove non-coding RNA genes and single exon genes. Next, the homology search by BLAST was performed against nr for finding the orthologs in different species. Finally, we deduced complete gene structure using comparative genomic analysis and removed some genes with known motifs in the novel coding sequence. This series of analysis provided with a list of 1,600 KAO-NASHI genes.

In order to unveil these KAO-NASHI genes, we decided to use Medaka (*Orzias latipes*) as a model animal because it is easy to obtain embryos and to observe the organs or anatomical structure. The gene knockdown method using morpholino-antisense-oligonucleotides (MO) is especially effective for Medaka embryos. We have so far identified medaka orthologs for over 60% of human KAO-NASHI genes using the medaka EST and whole genome shotgun sequence databases. We will present the data regarding expression profiles of these KAO-NASHI genes at different developmental stages and phenotypes generated by knockdown analysis.

Effects of C-propeptide Mutations in Type I Collagen on Extracellular Matrix Deposition and Fibrillogenesis.

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Mutations in the C-propeptide region of Type I collagen have been found in patients with Type II-IV Osteogenesis Imperfecta (OI). We identified four novel C-propeptide mutations by RT-PCR and direct sequencing of COL1A1 cDNA. Three involved substitutions at conserved residues, including W1097C (nt 15408) in Type III OI, and T1120I (nt 15476) and P1266H (nt 16331) in Type IV OI. The fourth proband, with Type II OI, had a six nt deletion at the E51/I51 junction that caused the in-frame insertion of all but two nt of I51 in cDNA and the loss of the 7th C-propeptide cysteine. We compared proband fibroblasts with normal control cells, a proband with a C-terminal helical mutation (Type III OI, 1(I) G997S) and a published lethal C-propeptide mutation (D1263Y, nt 16321; Pace, J Med Gen 2002; 39:23). All mutant collagens, including G997S, showed broad backstreaking of 1(I) on SDS-Urea-PAGE of steady state collagen, suggesting delayed chain incorporation. Incorporation of pro-1 chains with single amino acid substitutions was 2-3 times longer than in control cells (~20 minutes). D1263Y and helical G997S procollagen required 4 times longer to incorporate 1 chains than control, and pro-1 chains with an intronic insertion were only 1/3 incorporated at 120 minutes. A processing assay suggested delayed C-propeptide removal from secreted collagens containing substitutions in the C-propeptide or at the end of the helix. Overmodified collagens are incorporated into fibroblast matrix in culture and form mature cross-links. Skin and bone fibrils from two probands (T1120I and P1266H) were examined by electron microscopy (EM). Proband dermal fibrils are approximately 10-15% larger than control fibrils. SEM revealed increased disorganization of bone fibrils, compared to an OI proband with a mid-helical mutation. The data show that alterations in the C-propeptide or in interacting regions lead to delayed procollagen processing, incorporation of overmodified collagen into fibrils, and altered fibril organization.

Genetic interaction of TRPS1 and RUNX2 during skeletal development identified by SNP analysis of the RUNX2 promoter. *D. Napierala*¹, *R. Morello*², *G. Zhou*², *Q. Zheng*², *T. Bertin*², *R. Shivdasani*³, *B. Lee*^{1,2}. 1) HHMI, Baylor College of Medicine, Houston, TX; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA.

TRPS1 and *RUNX2* are transcription factors important in skeletal development. Mutations in *TRPS1* cause tricho-rhino-phalangeal syndrome (TRPS), while loss of function mutations of *RUNX2* lead to cleidocranial dysplasia (CCD). Both TRPS and CCD patients exhibit short stature, brachydactyly and cone-shaped epiphyses suggesting that TRPS1 and RUNX2 may play critical roles during chondrocyte differentiation. RUNX2 is a master transcriptional regulator of osteoblast differentiation and chondrocyte maturation. Its expression, however, must be downregulated in mesenchymal condensations during endochondral ossification. Until now, transcriptional repressors of Runx2 *in vivo* have yet to be identified. By combining SNP analysis of control and CCD subjects and cross species sequence analysis, we have identified conserved GATA domain binding sites in the RUNX2 promoter. Because TRPS1 is the only GATA domain transcription factor known to function in skeletogenesis, we analyzed its expression pattern during mouse embryonic development. Interestingly, *Trps1* is expressed in skeletal regions where *Runx2* needs to be turned off, including all mesenchymal condensations destined for chondrogenic lineages. Moreover, TRPS1 specifically binds to the GATA cis elements found in the RUNX2 promoter. In transfection studies, *Trps1* strongly represses the *Runx2* promoter as well as the Runx2 proteins transactivation of its target promoters. Deletion analysis suggests that the interaction is mediated by the RUNT domain. Finally, transgenic mice overexpressing *Trps1* exhibit lengthened growth plates. Together, our data suggest that *Trps1* can inhibit expression of the *Runx2* gene as well as *Runx2* protein function both early during cell fate commitment of mesenchymal condensations and later during chondrocyte maturation. This genetic and biochemical interaction explains some of the clinical features found common in TRPS and CCD.

Activation of Phospholipase D in Angiomyolipomas from Tuberous Sclerosis Complex (TSC) Patients. *K.-S. Au, R. Zhu, H. Northrup.* Dept Pediatrics, Univ Texas Medical Sch, Houston, TX.

Tuberous sclerosis complex (TSC) is a dominantly inherited disease in humans affecting 1:6700 live births. Affected patients are at risk to develop hamartomas in many tissues and organ systems. The greatest morbidity is caused by renal and CNS complications. Renal angiomyolipomas (AMLs) and cysts are common occurring in >80% of patients. The disease is caused by mutations in one of two genes (TSC1 and TSC2) that code for the tumor suppressing proteins hamartin and tuberlin, respectively. Functions of hamartin and tuberlin in normal cells have been actively studied over the last decade concluding that the hamartin/tuberlin complex functions as a GTPase activating protein (GAP) modulating signals from small GTPase (i.e. Rheb, Rap1A, Rab5) to regulate protein translation, vesicle trafficking, cell survival, migration, and proliferation events. The functions involve several cell-signaling pathways including but not limited to PI3K/Akt, Wnt/GSK3, Rho/ERK. Through comparative gene expression microarray studies using mRNAs isolated from three kidney AMLs of a TSC patient with a known germline TSC2 mutation, we observed elevated expression of G-protein coupled receptors and phospholipase D (PLD). The finding was observed in all three samples. The tumors studied were previously reported to have loss of heterozygosity of the TSC2 gene suggesting that a new signaling event in these tumors was activated with the absence of functional tuberlin. Immunoblotting studies demonstrated elevated PLD proteins present in two of the tumor lysates that showed the most elevated PLD1 mRNA by microarray studies. Elevated PLDs have been observed in many tissues studied from human cancers. Of particular importance, elevated PLDs were correlated with reduced sensitivity to rapamycin treatments in breast cancer patients. Rapamycin is currently under study in a clinical trial to treat kidney AMLs in TSC patients. PLD activity in TSC patients may be important in determining their response to rapamycin efficacy for treatment of TSC tumors. Genetic variants leading to higher PLD activity may modify the disease phenotype and tumor treatment outcome for TSC patients.

Pcp4: construction and validation of a transgenic mouse model. *G.M. Barlow¹, J.M. Vesa¹, M. Brennan², D. Patterson², J.I. Morgan³, J.R. Korenberg¹*. 1) Med Genetics Institute, Cedars-Sinai & UCLA, Los Angeles, CA; 2) Eleanor Roosevelt Institute, Denver, CO; 3) St Judes Childrens Hospital, Memphis, TN.

Pcp4 is a calmodulin-binding IQ motif peptide that modulates apoptosis and the activity of CaMKinase II, which is important for long-term potentiation (LTP), learning and memory. It is expressed in neuronal subpopulations of the cortex, dentate gyrus granule cells, hippocampal pyramidal CA2 neurons, cerebellar Purkinje cells, thalamus, caudate putamen and septal nuclei. In vitro, cells transfected with Pcp4 exhibit reduced UV-induced apoptosis and neuronal populations expressing Pcp4, including dentate gyrus granule cells and cerebellar Purkinje cells, can exhibit resistance to degeneration. The Pcp4 gene maps to 21q22.2, which contributes the neurocognitive features of Down syndrome (DS). Further, whilst DS individuals exhibit an increased risk of Alzheimers disease, it is unclear whether they exhibit the dementia expected from their neuropathology. Overexpression of Pcp4 may ameliorate the neural features of DS. We now report generation and validation of a transgenic mouse model for Pcp4, using a human BAC spanning the Pcp4 coding region and 84 kb upstream of the transcriptional start. Two independent lines of mice were shown to express the human Pcp4 mRNA. We then validated the model at the RNA, protein and histological levels as a test of neuron-specific DS features. Using Quantitative Real-time PCR with human and mouse gene-specific primers vs GAPDH controls, we found human Pcp4 mRNA levels 1.4 X those of the native mouse gene for Line 1, and 50-65 X for Line 2 (3 transgenics & 3 wildtypes for each of 2 litters/line). Western blot analyses of adult brain protein confirmed increased Pcp4 levels in Tgs vs wts. Using IHC, we demonstrated that adult neuronal distributions of Pcp4 in the Tg were similar to those seen in the wildtype. This mouse model will be useful for studying the potentially neuroprotective effects of increased Pcp4 expression and its effects on hippocampal learning and memory in DS.

Analysis of *BDNF* expression in rats treated with Semax and PGP under the brain experimental ischemia. L. Dergunova¹, V. Stavchanskii¹, A. Botsina², I. Platonova², T. Tvorogova², E. Shaykhutdinova¹, V. Skvortsova², S. Limborska¹. 1) Dept Human Molecular Genetics, Inst Molecular Genetics, Moscow, Russian Federation; 2) Dept of Basic and Clinical Neurology and Neurosurgery, Russian State Medical University, Moscow, Russian Federation.

Brain-derived neurotrophic factor (BDNF) is a natural neuroprotector involved in proliferation, differentiation and survival of neuronal and glial cells. It was revealed that *BDNF* expression was increased in rat cultured glial cells under treatment by the neuroprotector Semax (synthetic polypeptide Met-Glu-His-Phe-Pro-Gly-Pro; its N-terminus represents a fragment of adrenocorticotrophic hormone. We have analyzed the effect of Semax and its C-terminal fragment Pro-Gly-Pro (PGP) upon *BDNF* expression within cerebellum and forebrain cortex of the rats subjected to global brain ischemia. After 15 minutes of irreversible bilateral common carotid artery occlusion the animals were subjected to intraperitoneal injection of Semax, PGP or 0,9% NaCl. Animals were decapitated 30 minutes / 1 hour / 2 hours after the operation. Within the last group (2 hours) the animals were injected for the second time 1 hour after the occlusion. Sham-operated animals were used as a control. *BDNF* mRNA expression level was assessed by semi-quantitative PCR. *BDNF* PCR signals were normalized to the corresponding RT product GAPDH signal. BDNF and GAPDH PCR signals were analyzed for two cDNA samples of each rat. Under experimental conditions, the sham-operated and ischemized rats treated with 0,9% NaCl did not demonstrate the difference between *BDNF* expression levels within investigated brain regions. *BDNF* expression within the cerebellum of the ischemized rats also was not influenced by Semax and PGP. At the same time, comparing to the ischemized animals treated with 0,9% NaCl, expression level of *BDNF* within forebrain cortex of the animals treated with Semax and PGP was increased. Within the forebrain cortex of the animals treated with Semax and PGP *BDNF* expression level was found to be maximal 30 minutes after occlusion. Possibly, therapeutic effect of Semax is mediated by the increased production of *BDNF* within brain cells.

Polyalanine tracts of the ARX homeobox gene, mutation, conservation and function. *J. Gecz^{1,2}, O. McKenzie¹, M. Finnis¹, T. Fullston¹, C. Shoubridge¹, D. Cloosterman^{1,2}, M. Mangelsdorf^{1,2}*. 1) Dept of Genetic Medicine, Women's Department of Genetic Medicine, Womens & Childrens Hospital, Adelaide, Australia; 2) Department of Paediatrics, University of Adelaide, Adelaide, Australia.

Aristaless related homeobox gene, ARX is mutated in various forms of intellectual disability with or without additional features including infantile spasms, dystonia, lissencephaly, autism, dysarthria, etc. Results of our screening (>500 samples) indicate that mutations of ARX are found in virtually all families with non-syndromic mental retardation (NSXLMR) mapping across the ARX gene (8 out of 8 tested, 3 not tested yet; ~11% of published NSXLMR) and ~3.4% of putative XLMR (include brother pairs, trios, uncle-nephew pairs, cousin pairs and small unmapped families). So far the most frequent and the most pleiotropic ARX mutation is a duplication of 24bp (428-451dup24bp), which accounts for ~30% of known ARX mutations. It causes expansion of one of four polyalanine tracts of the ARX gene from 12 to 20 alanines. The function of polyalanine tracts, which are frequently found in transcription factors, is not known. We have studied various full-length and truncated, normal and mutated ARX constructs in transcription repression studies. Our results show that ARX is a strong repressor. We mapped this activity within one, highly conserved, invariable polyalanine tract, tract 4, which has not yet been found mutated in patients. The transcription repression activity of ARX is also affected by the 428-451dup24bp (tract 2) and (GCG)₁₀₊₇ (tract 1) polyalanine tract expansion mutations, which were tested individually or in the context of the full length ARX. There is a correlation between the number of extra alanine residues and the resulting transcription repression. Deletions within alanine tracts 1 and 2 have little effect on the repression activity. Stably transfected PC12 cells (tet-on) with normal and 428-451dup24bp ARX full-length cDNAs do not show signs of aggregation of ARX. In summary, the dup24bp mutation is the most prevalent and the most pleiotropic ARX mutation, which likely exerts its pathogenic effect via elevated repression activity of the mutated ARX protein.

Connexin 32 promoter P2 mutations highlight a common mechanism of demyelination. *M. Girard*¹, *H. Houlden*², *C. Cockerell*^{3,4}, *D. Ingram*³, *NW. Wood*², *M. Goossens*¹, *RWH. Walker*^{3,4}, *MM. Reilly*². 1) Genetique Moleculaire et Physiopathologie, INSERM U468, Hopital Henri Mondor, France; 2) Department of Molecular Neurosciences, Institute of Neurology, The National Hospital for Neurology and Neurosurgery, London, UK; 3) St Bartholomews Hospital and The Royal London Hospital, London, UK; 4) Princess Alexandra Hospital, Harlow, Essex, UK.

Charcot-Marie Tooth disease (CMT) is the most common hereditary disorder of the peripheral nervous system. The X-linked form of CMT is mainly caused by mutations in the GJB1 gene, encoding the gap-junction protein Connexin 32 (Cx32). The number of different mutations reported in the coding region of this gene is now over 250, however only two mutations have been identified in the non-coding regions of Cx32. Here, we identified a large family from Essex in the UK with CMT. Sequencing of the Cx32 promoter region revealed a novel mutation at position -526bp in the P2 region, which segregated with the disease and was not present in controls. To prove pathogenicity, linkage analysis was carried out using high-density markers on the X-chromosome. This revealed highly significant genetic linkage to the Cx32 (Xq13.1) region with no recombination between marker DXS993 and DXS990. The Connexin 32 P2 promoter region was sequenced in a number of different species and the region of interest was conserved throughout. This mutation is located in a highly conserved SOX10 binding site, and functional studies carried out on the mutant Cx32 promoter revealed that this mutation reduced the activity of the Cx32 promoter and the affinity for SOX10 binding. These data and previous observations suggest that mutations in this region of the Cx32 gene are part of a complex mechanism of Schwann cell dysfunction involving SOX10 and EGR2 and leading to demyelination. Progress in the understanding of how mutant genes lead to the development of different types of neuropathy is likely to be critical to understanding the pathogenesis of CMT.

In vivo inhibition of caspase-6 cleavage of expanded huntingtin protects against neurodegeneration. RK.

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Huntington disease is a neuropsychiatric disease caused by expansion of a trinucleotide repeat in the HD gene, huntingtin. Proteolytic cleavage of huntingtin by caspases with concomitant generation of toxic huntingtin fragments has been well characterized in vitro, and caspase-mediated fragments of huntingtin are present in HD brain prior to clinical onset of HD. These observations provide strong support for the toxic fragment hypothesis which proposes that huntingtin cleavage is a crucial determinant of pathology in HD and furthermore suggest that caspase-resistant (CR) huntingtin may delay or prevent the onset or progression of HD in vivo. To test this hypothesis using the YAC animal model of HD, we used homologous recombination to mutate both caspase 3 and 6 cleavage sites within full length genomic huntingtin with 15 and 128CAG. Neuropathological analysis revealed that complete inhibition of caspase cleavage of expanded htt protects against the neurodegeneration observed in the YAC 128 model. We then sought to determine whether caspase 3 or caspase 6 is the key player in generating the selective toxicity observed in the striatum. Analysis of 128C3R and 128C6R lines demonstrated that while 128C3R did not provide protection, selective mutagenesis of the caspase-6 site provided significant protection from neurodegeneration in vivo. In two independent 128C6R lines, brain weights and striatal volumes are indistinguishable from wild-type. In contrast, brain weights and striatal volumes in caspase cleavable YAC128 mice are decreased by 10 (*p<0.05) and 16% (**p<0.0003) respectively. These results suggest that caspase-6 cleavage of huntingtin may play an important role in the generation of specific toxic fragments resulting in selective neurodegeneration in HD and that preventing caspase-6 cleavage of huntingtin may be a promising therapeutic strategy.

ALS2 gene product expression pattern in mouse tissues. *F. Gros-Louis¹, R. Devon², P. Orban², J. Laganier¹, P. Hince¹, M.R. Hayden², G.A. Rouleau¹.* 1) McGill University Health Centre, Montreal General Hospital, Montreal, Quebec, Canada; 2) Centre for Molecular Medicine and Therapeutics, University of British Columbia, Vancouver, Canada.

Amyotrophic lateral sclerosis (ALS) is a devastating disorder that has an incidence of between 0.5 and 3 per 100,000. ALS is characterized by the degeneration and death of motor neurons in the cerebral cortex, brainstem and spinal cord, resulting in progressive muscle weakness, atrophy and death from respiratory paralysis. Recently, the gene responsible for a juvenile onset autosomal-recessive form of ALS, ALS2 was identified. The ability to detect the ALS2 gene product (alsin) with an antibody will be an important resource for the to study tissues expression pattern in mouse and molecular pathways that lead to motor neuron degeneration in ALS. Six oligopeptides (20mers) have been generated that are specific to either or to both the published splice variants. The peptides have been designed against specific regions that are meant to be antigenic according to the hydrophilicity plot . All our antibodies have been generated in rabbits using standard methods. Immunohistochemistry and western blotting were performed using standard methods. We demonstrated that alsin is widely expressed during development at low levels when compared to adult mouse. The protein is present in the cell bodies of various tissues, including neurons of the cortex, brain stem and spinal cord. Surprisingly, the highest level of expression was in the cytoplasm of the cerebellar Purkinje cells and in the inferior olivary nucleus of the brain stem, regions not previously known to be affected in ALS2. The selective loss of motor neurons, in the face of a widespread expression, suggests that alsin may have a pivotal role in maintaining the integrity of this cell population. These data also suggest that alsin may have different functions during development and adulthood. It is not clear whether or not the cerebellum is involve in the pathology of ALS as motor neuron degeneration may mask any cerebellar phenotype but an understanding of cerebellar involvement may lead to important new insights in the molecular pathogenesis of ALS.

Functional analysis of hamartin and tuberin using yeast-two-hybrid screening. *S. Khare, A. Kumar.* MRDG, Indian Institute Of Science, Bangalore, Karnataka, India.

Tuberous sclerosis complex is an autosomal dominant genetic disorder characterized by the presence of benign tumors called hamartomas in multiple organ systems, particularly in brain, skin, lungs, heart and kidneys. TSC is caused by mutations in two distinct genes: TSC1 located on chromosome 9q34 and TSC2 located on chromosome 16p13.3. TSC1 encodes a protein hamartin of 130-kDa, which contains a putative transmembrane domain and two putative coiled-coil domains (CCDs). A stretch of 133 amino acids (from a.a. 145-278) close to the potential transmembrane domain is conserved in human, mouse, rat and *Drosophila*, suggesting an unknown functional role for this stretch. TSC2 encodes tuberin, a 180-kDa protein (1,807 amino acids), which contains two coiled-coil domains, two transactivation domains, a calmodulin binding domain and a GAP domain. It has been shown that tuberin and hamartin interact with each other and form a complex *in vivo*. This interaction is mediated by N-terminal CCD (N-CCD) of tuberin and C-terminal CCD of hamartin. However, the function of C-terminal CCD of tuberin is not known. We have used yeast-two-hybrid system to identify the interacting proteins for the 133 amino acids long stretch of hamartin as well as C-CCD of tuberin. For this purpose, the 133 amino acids long stretch of hamartin and the C-CCD of tuberin were cloned separately in Gal4 DNA binding domain (DNA-BD) vector pGBT9 and used as baits to screen a fetal mouse brain cDNA library cloned in Gal4 activation domain (AD) vector, pACT2. Yeast-two-hybrid library screening was carried out using 1×10^6 independent cDNA clones for each bait. After the screening, the transformants were assayed for interaction using nutritional selections, - and -galactosidase assays. Following screening, we have identified putative interacting proteins for the 133 amino acids long stretch of hamartin as well as C-CCD of tuberin. We are in the process of performing co-immunoprecipitation to confirm the interactions. (Financial support from the CSIR, New Delhi is gratefully acknowledged).

Subnuclear localization of ATRX with PML and DAXX: identification of domains and effect of mutations. M.

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ATRX encodes a SNF2-like chromatin remodeling protein, mutations in which cause X-linked mental retardation (XLMR). Although most mutations lie within the chromatin interaction domains little is known about how these contribute to *ATRX* function and normal CNS development. Recent studies have shown that *ATRX* forms a complex with DAXX and associates with PML oncogenic domains (PODs) suggesting that it may regulate the formation of nuclear domains or the expression of target genes. We define *ATRX* domains essential for subnuclear localization and assess how patient mutations or loss of *ATRX* impinge on the formation of such domains. Full-length *ATRX* and a panel of deletion constructs were fused to GFP to identify regions responsible for the correct localization of the protein. The full length protein maintains the endogenous pattern characterized by speckles amongst diffuse nuclear staining. *ATRX* fragments highlighted two regions responsible for speckle formation; L2 (amino acids 924-1651) and L19 (1965-2492). L19 but not L2 colocalized with PML and DAXX suggesting that the C-terminal region directs *ATRX* to PODs. Mutations in L19 attenuated the association of *ATRX* with PODs and also reduced their number. To explore this *in vivo* we examined the localization of DAXX in a forebrain-specific *ATRX* knockout mouse model. We show by immunohistochemistry that DAXX also co-localizes with *ATRX* in mouse. However in the forebrain of the *ATRX* knockout animals, DAXX was mislocalized and speckles were no longer observed. Our results have identified regions of *ATRX* that are important for its localization to PODs and mutations alter this association. Moreover, an absence of *ATRX* similarly effects the localization of DAXX. Taken together these results suggest that altered subnuclear localization of *ATRX* may contribute to the XLMR phenotype.

Defining new genes in Xq28 distal region as candidates for linked SNC diseases. *M.G. Miano¹, J. Monfregola¹, C. Laperuta¹, S. Crispi², D. Esibizione¹, N. Silva¹, M. D'Urso¹, M.V. Ursini¹.* 1) Institute of Genetics and Biophysics Adriano Buzzati Traverso, CNR, Naples, Italy; 2) BioGeM, Ariano Irpino, Italy.

The human Xq28 region contains several disease loci whose causative gene is still unknown. However, the difficulty to isolate new disease genes is remarkable because of the paucity of polymorphic markers available for linkage study. Our aim was to refine critical regions for SNC diseases mapping in Xq28 such as MRX72, PMGX, and Waisman syndrome. Starting from the nucleotide sequence, partially performed in our laboratory, we have selected new DNA markers. We identified several new STRs that have high allele frequencies in Caucasian population for which we have established the index of heterozygosity and consequently their power in linkage studies. Those STRs are in use to narrow down disease loci for SNC diseases in affected families identified by us in collaboration with clinical geneticists. Accordingly, this strategy is in use to define the linkage interval for another X-linked mental retardation (MRX81) that was recently identified and mapped by our group. Among the Xq28 disease genes, we focalized on few genes with a putative role in the SNC development. Briefly, we identified a novel gene closed to F8c, in tight linkage with MRX72 locus, which encodes an evolutionary conserved protein containing three putative transmembrane motifs, appearing to be a novel member of a large family of proteins. In the second instance, we studied the gene encoding for a key enzyme of the carnitine biosynthetic whose physiological relevance in neuronal survival and differentiation is documented. Finally, the third gene of interest for our group is located less than 10 kb from the telomere associated repeat of the X chromosome which from bioinformatic and functional analysis resulted to be a G-actin binding protein which has a clear well documented role cytoskeleton organization and in neuronal cell migration. Functional, structural and evolutionary data for all of them will be illustrated in more details according with an approach to understand gene function and to correlate in human diseases.

NIPA1, mutated in spastic paraplegia type 6 (SPG6), is a putative neuronal endosomal transporter. *R.D. Nicholls, K. Talbot, Q-C. Yu, J-H. Chai.* University of Pennsylvania, Philadelphia, PA.

SPG6 is a hereditary spastic paraplegia characterized by corticospinal tract degeneration and progressive lower-extremity spasticity. In this disorder, we previously found a dominant-negative mutation in *NIPA1*, a novel gene from human chromosome 15q11.2 (AJHG 73:898-925, 967-71). Two mechanisms may account for brain-enriched *Nipa1* expression, transcriptional regulation of the *Nipa1* promoter and/or a non-coding antisense transcript (*Nipa1as*) that overlaps *Nipa1*. *Nipa1as* is alternatively spliced and initiates from a bidirectional promoter shared with the adjacent *Nipa2* gene, but has much lower expression levels and could serve to regulate levels of *Nipa1* in brain or other tissues. To investigate the function of NIPA1, which encodes a putative transporter with 9 predicted transmembrane (TM) domains, we generated antisera against peptides from the TM4-TM5 loop and the C-terminus, then examined the expression and subcellular localization of NIPA1. Western blot studies of total and membrane protein fractions from mouse tissues and *Nipa1-EGFP* transfected HeLa cells show that both endogenous NIPA1 and NIPA1-EGFP fusion protein are enriched in the TM fractions. Interestingly, a short isoform of NIPA1 is identified only in the brain, suggesting brain-specific processing. Immunohistochemical studies demonstrate that NIPA1 is widely expressed in the cytoplasm and dendrites of neurons in many regions of the human and mouse nervous system, such as the cerebral cortex, hippocampal formation, cerebellum, midbrain, pons and medulla. NIPA1 is also expressed in oligodendrocytes of the cerebral cortex and corpus callosum, as well as in Schwann cells of peripheral nerves. Although a NIPA1-EGFP fusion protein overexpressed in HeLa cells is present in cytoplasmic vesicles co-localized with -Lamp1, a late endosomal/lysosome marker, electron microscopy on mouse cerebellar Purkinje cells shows that NIPA1 *in vivo* is present in clusters along the endoplasmic reticulum and adjacent vesicles possibly representing early endosomes. Further studies will examine co-localization with endosomal markers and the potential transport function of NIPA1 in regulating vesicular trafficking.

Identification of distal elements regulating *Smn* gene expression. C. Rochette¹, A. Semionov¹, N.L. Germain¹, F. Vigneault², R. Drouin², L.R. Simard¹. 1) Centre de recherche de l'Hôpital Sainte-Justine, Université de Montréal, Montréal, QC, Canada; 2) Centre de recherche CHUS, Sherbrooke, QC, Canada.

There exist two survival motor neuron (*SMN*) genes in humans, the result of a 500-kb duplication that occurred after the divergence of lineages leading to humans and mice. Mutations in the *SMN1* gene are responsible for childhood spinal muscular atrophy (SMA) while ablation of the single mouse *Smn* gene causes early embryonic lethality. Up-regulation of the human *SMN2* gene is an important target for the treatment of SMA. We have previously characterized the minimal *SMN*/*Smn* promoters and have now screened the entire *Smn* gene, which is half the size of its human counterpart, for distal elements regulating transcription. We performed an inter-species sequence comparison using PIPMaker, generated a series of reporter gene constructs spanning the entire gene and conducted *in vivo* genomic footprinting experiments to screen for DNA-protein interactions within specific genomic segments. Sequence conservation between the 30 kb human *SMN* and 14 kb mouse *Smn* genes was very sparse outside the coding regions. Only two segments displayed 75 identity of 100 bp, the minimal promoter region and within the 5'-most portion of intron 1. An additional segment in intron 5 showed 70 identity of 100 bp. While two genomic fragments exerted a small effect on TK promoter activity, only one of these was found to regulate the *Smn* promoter *in vitro*. Various reporter-gene constructs that contained the minimal *Smn* promoter and increasingly larger fragments of intron 1 displayed an incremental silencer effect. Inclusion of 520 nt of intron 1 caused a 16-fold decrease in *Smn* promoter activity in transient transfection assays of P19 cells. This effect was orientation independent and was replicated in differentiated P19, NSC34, SK-N-SH and HepG2 cells. *In vivo* DNA footprinting of P19RA cells using 3 different DNA modifying agents failed to reveal any convincing DNA-protein interactions involving this region. We are currently testing the hypothesis that the observed silencer effect is most likely mediated via a mechanism involving secondary structures within *Smn* intron 1. *Funded by CIHR.*

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Human spastin (SPG4): Functional characterization of potential nuclear export sequences. *J. Schickel, C. Beetz, T. Deufel.* Institut fuer Klinische Chemie und Laboratoriumsdiagnostik, Klinikum der Friedrich-Schiller Universitaet Jena, Erlanger Allee 101, 07740 Jena, Germany.

Mutations in the human spastin gene (SPG4) cause the most prevalent form of autosomal dominant hereditary spastic paraplegia (HSP), a neurodegenerative disorder characterised by progressive weakness and spasticity of the lower limbs. In a recent study using polyclonal antibodies against the N-terminal spastin sequence we have shown that the native protein is localised in both the perinuclear cytoplasm and the nucleus. Furthermore, using a reporter system based on four in-frame fused copies of green fluorescent protein we demonstrate that spastin carries two nuclear localisation sequences both independently functional in mediating nuclear entry. We suggest a dual function for the spastin protein. Involvement in cytoplasmic trafficking and a still unknown function in the nucleus. Precise lokalisation may be a regulated process involving import into the nucleus as well as export back into the cytoplasm. Supporting this notion, two potential nuclear export sequences are found within the spastin amino acid sequence. Our study was aimed at characterizing domains in the N-terminal part of spastin impeding nuclear entry of transiently expressed spastin in cultured cells. First results identified a short sequence motif of approximately 25 amino acids in size to be responsible to retard spastin in the cytoplasm of the cell.

Neural intranuclear inclusions in FMR1 premutation carriers affected by fragile X-associated tremor/ataxia syndrome (FXTAS) contain FMR1 mRNA. *F. Tassone, C. Iwahashi, P.J. Hagerman.* Department of Biochemistry and Molecular Medicine.

We have recently identified a neurological disorder among male carriers of the fragile X premutation, characterized by tremor and/or ataxia, cognitive deficits, parkinsonism, and autonomic dysfunction. The disorder, named fragile X-associated tremor/ataxia syndrome (FXTAS) has been observed almost exclusively in older adult premutation males. Our studies have indicated that at least 30% of male carriers over 50 years of age will develop neurological symptoms of FXTAS. Post-mortem studies of the brains of individuals who had FXTAS have identified ubiquitin-positive intranuclear inclusions in both neurons and astrocytes throughout the cerebrum and brainstem of all cases examined to date (n = 11). Although neuronal inclusions have been observed in other trinucleotide repeat disorders including Huntingtons disease, Kennedys disease, and a number of the spinocerebellar ataxias, the presence of inclusions in FXTAS is puzzling, since the FMR1 protein is structurally normal, and present in near-normal levels, in premutation carriers. Because premutation carriers possess elevated levels of FMR1 mRNA, we have hypothesized that FXTAS might result from a toxic gain of function of the FMR1 mRNA itself. The mechanism by which increased FMR1 mRNA levels lead FXTAS and to inclusion formation is not fully understood at present. By analogy to the RNA gain-of-function model in myotonic dystrophy, the abnormal FMR1 mRNA could trigger inclusion formation by sequestering CGG binding proteins, which may result in loss of their normal functions. This hypothesized sequestration may also lead to the neuropathology and clinical features of FXTAS. Here, we report that the FMR1 mRNA is present within the intranuclear inclusions supporting the direct involvement of the FMR1 mRNA in the inclusion formation observed in FXTAS.

***MOB* is a gene encoding human sphingomyelin synthase 1.** I. Vladychenskaya, L. Dergunova, V. Dmitrieva, S. Limborska. Dept Human Genetics, Inst Molecular Genetics, Moscow.

Prior investigation of human brain cDNA libraries revealed an evolutionarily conserved gene *MOB* that has been cloned in silico on chromosome 10. Analysing hypothetical primary structure of the transmembrane peptide product proposed for the *MOB* major transcript we have found it to be identical with the primary structure of human sphingomyelin synthase 1 (SMS1); now we consider *MOB* as a gene encoding SMS1. In human, two yet uncharacterized genes paralogous to *MOB* (*SMS1*) were revealed. These genes termed p1 *MOB* and p2 *MOB* are localized on chromosomes 4 and 10, respectively; together, they represent a novel gene family. *MOB*, p1 *MOB* and p2 *MOB* orthologs of conserved genomic structure were found in warm-blooded animals; orthologs of p2 *MOB* were also found in *D. melanogaster* and *C. elegans*. We have examined *MOB* (*SMS1*) transcription activity; expression levels of the major *MOB* transcript (transcript encoding SMS1) were assessed among different human tissues by means of semi-quantitative RT-PCR. Comparing to the maximal expression level detected within the brain tissues, transcript abundance within kidney is one and a half times less, within lung and liver - two times less, within spleen and lymphatic node - seven times less. We have also analysed the expression pattern of the alternative *MOB* transcript lacking the longest coding exon VII and found it to be similar with that of the major transcript.

Molecular characterization of the mammalian ISWI gene, *SNF2L*, and its assessment as an X-linked Mental Retardation (XLMR) candidate gene. *D.J. Yip^{1, 2}, A.C. De Maria¹, M.A. Lazzaro¹, P. Lavigne¹, D. Vallée¹, D.J. Picketts^{1, 2}.* 1) Molecular Medicine Program, Ottawa Health Research Institute, Ottawa, Ontario, Canada; 2) Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Ontario, Canada.

Chromatin remodeling is an ATP-dependent process performed by an assortment of multi-protein complexes each of which contains a SWI/SNF-like catalytic subunit. Mutations that have been observed in genes encoding chromatin-remodeling proteins have been linked to developmental disorders. Moreover, mutations in SWI/SNF homologs, such as ATRX, have been shown to cause X-linked mental retardation (XLMR), as a result chromatin-remodeling factors represent strong XLMR candidates. In *Drosophila*, ISWI functions as the ATP-hydrolysing component in chromatin modulating complexes important as regulatory mechanisms for developmental gene expression. The two mammalian ISWI orthologs, *SNF2H* and *SNF2L* present differential expression patterns suggesting that they possess distinct developmental roles. In order to assess these genes as candidates for human disorders we have characterized *SNF2L* as a brain-enriched transcript with increased expression upon differentiation, suggesting a role in maturing neuronal cell populations. We have shown an increase in *SNF2L* expression across a 96-hour differentiation timecourse in mouse NIE115 and human SH-SH5Y neuroblastoma cells by RT-PCR and indirect immunofluorescence. The *SNF2L* gene spans 77 kb and is encoded by 25 exons that undergo alternative splicing. Isoforms are generated by the alternate use of exons 1 and 13, and by alternate donor splice sites within exon 24. Interestingly, alternative splicing within exon 24 removes the nuclear localization signal and alters the subcellular distribution of *SNF2L*. *SNF2L* localizes to Xq25-26, a 7cM region of the X chromosome where several mental retardation disorders overlap. We identified 3 non-coding nucleotide polymorphisms in the *SNF2L* gene after screening 12 XLMR families for which the disease gene was localized to this genomic interval. Despite the absence of a pathogenic mutation, *SNF2L* remains a prime candidate for XLMR localized at Xq25-26.

Identification of genes involved in the pathogenesis of haemangiomas using suppression subtractive

hybridization. *A.J Scott¹, K. Robertson², R. Crawford¹, A. Smith³, D. Marchuk⁴, P. North⁵, J. Pearson⁶, R. Roberts¹, J.N. Berg^{1,2}.* 1) Department of Medical and Molecular Genetics, Kings College London, Guy's Hospital, UK; 2) Department of Molecular and Cellular Pathology, Ninewells Hospital and Medical School, Dundee, UK; 3) Department of Academic Surgery, Kings College London, St Thomas's Hospital, UK; 4) Department of genetics and microbiology, Duke University Medical Centre, Durham, NC; 5) Arkansas Childrens Hospital, Little Rock, AR; 6) Centre for Cardiovascular Biology and Medicine, Kings College London, UK.

Infantile haemangiomas are benign tumours of vascular endothelial cells. They grow rapidly after birth and proliferate to form a network of blood capillaries, followed by apoptosis of endothelial cells and involution. Haemangiomas have been shown to be monoclonal and are thought to arise following somatic mutation in endothelial cells. Haemangiomas also provide a model of angiogenesis. Previous gene expression studies in haemangiomas have focused on a pre-selected group of genes. Through suppression subtractive hybridisation, an unbiased technique, this project has identified a number of genes that may be involved in infantile haemangioma formation. Real-time quantitative PCR has confirmed differential expression of these genes in infantile haemangioma tissue compared to that of normal endothelial cells in culture. Expression profiles show up-regulation of Decorin and Ribonuclease 1 and down-regulation of chemokine (CXC) receptor 4 and connective tissue growth factor receptor. These proteins are known to be expressed by endothelial cells or to affect angiogenesis in culture but have not before been implicated in the pathogenesis of infantile haemangioma. Immunohistochemistry has confirmed the expression of ribonuclease 1 in haemangioma endothelial cells in a proportion of infantile haemangiomas. For Decorin and CXCR4, staining showed high levels of protein expression in connective tissue, but less prominent staining around haemangioma endothelial cells. Our data suggest that the role of these genes in both the control of endothelial cell function and in haemangioma formation merits further investigation.

TAZ mRNAs in Barth Syndrome subjects, alternative splicing and exon evolution. *I. Gonzalez.* Dept Res, Sr Res Scientist, DuPont Hosp for Children, Wilmington, DE.

Barth syndrome (MIM 302060) is an X-linked condition that includes dilated cardiomyopathy, neutropenia, failure to thrive, abnormal mitochondria, 3-methylglutaconic aciduria. The mutated gene, TAZ, was first described in 1996 and was reported to produce a large set of alternatively-spliced mRNAs with initiations of transcription upstream of exons 1 and 3. Since then, disease-causing mutations had been found in all exons except exon 5. Because of the initially-described 2nd initiation of transcription in intron 2, with in-frame initiation of translation in exon 3, we hypothesized that subjects with mutations in exons 1 and 2 would produce normal "short products" which might attenuate their phenotype. Moreover, it was of interest to determine which splice variants were functional, as exon 5 is not present in yeast and rodent TAZ genes and the variant lacking this exon is the most abundant in humans. We characterized TAZ mRNAs from a set of Barth syndrome subjects and of healthy controls. TAZ genes and mRNAs of primates were also included to determine the origin of exon 5. Our findings include: [1] There is only one site for initiation of transcription upstream of exon 1. [2] The alternatively-spliced assortment is more limited than previously thought. Our results suggest that only 2 functional protein variants exist, 5 and full length, in agreement with results of others who have tested functionality of TAZ minigenes. [3] There are two alternative splice sites within introns 1 and 2 which could potentially produce an in-frame product; their functionality has not yet been tested. [4] Although the exon 5 sequence is present in Old and New World monkeys, it lacks one of the splicing signals. It evolved into splicing-competent "exonhood" in the hominoid lineage, after the split from Old World monkeys. Its true exonic status is confirmed by the recent finding of an affected child with an exon 5 mutation.

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Analysis of emerin deficient mouse. *Y. Hayashi, R. Ozawa, M. Ogawa, I. Nishino.* Dept Neuromuscular Research, Natl Inst Neuroscience, NCNP, Tokyo, Japan.

Emery-Dreifuss muscular dystrophy is an inherited muscular disorder characterized by the triad of progressive weakness in humero-peroneal muscles, early contractures and cardiomyopathy with conduction block. We produced emerin lacking mice as a model of X-linked Emery-Dreifuss muscular dystrophy by insertion of a neomycin resistance gene into exon 6 of the gene including a transmembrane domain. Tissues from the mutant mice lacked normal mRNA and protein of emerin. However, the emerin deficient mice displayed normal growth rate indistinguishable from their littermates and fertile. The mutant mice did not show any joint contractures and gait abnormality. No muscle weakness or atrophy was observed, and they can survive more than 100 weeks. Histological analysis of cardiac and skeletal muscle from the mutant mice showed no dystrophic changes. Lamin A, lamin C, lamin B2 and LAP2 localized at nuclear membrane and showed no difference from heterozygous and wild type. This result suggests only deficiency of emerin did not cause muscular dystrophy in mice.

Haplotype-analysis of TNF and TNF polymorphisms and its relation to TNF expression *in vivo*. S. Schulz^{1,2}, T. Suess^{1,2}, D. Rehfeld^{1,2}, U. Schagdarsurengin², I. Hansmann², K. Werdan¹, U. Mueller-Werdan¹, C. Glaeser². 1) Dep Intern Med, Univ Halle, Halle, Germany; 2) Inst Human Genetics, Univ Halle, Halle, Germany.

TNF and TNF are important cytokines in the complex signalling pathway involved in the development of CAD. **Methods:** We studied the influence of two polymorphisms (PM) of TNF (c.-238G>A) and TNF (T60N) on the *in vivo* TNF-expression (RT-PCR: monocytes; ELISA: plasma) in the context of CAD. In this clinical study 176 age matched patients with angiographically confirmed diagnosis were included: 88 patients with severe CAD and 88 patients without any coronary symptoms as controls. **Results:** We could show that the c.-238G>A-PM is influencing TNF mRNA-expression significantly in the whole patient group (GG: 6.2±5.9ag/cell; AG: 8.8±6.2ag/cell; p=0.019). This influence is mainly due to variation in the CAD-patients (p=0.013). Furthermore, we could prove a significant effect of the T60N PM on TNF gene expression in the whole patient group (AA: 4.3±4; AC: 7.7±6.7; CC: 5.7±5.4ag/cell; p=0.007). However, this regulation was more pronounced in the control group (p=0.011) than in the CAD-group (p=0.079). A haplotype-analysis revealed that the c.-238G>A PM is a stronger predictor for an elevated TNF mRNA-expression. The heterozygous variant AC-AG (T60N-c.-238G>A) causes the highest expression (9±7.6ag/cell) followed by the variant CC-AG (8.4±2.8ag/cell). All other combinations showed significant lower expression rates (AC-GG: 7.5±6.6; CC-GG: 5.5±5.5; AA-GG: 4.3±4ag/cell; p=0.002). On translational level only the T60N-PM, could be referred as a predictor of TNF protein-expression in the group of CAD-patients but not in the whole patient group: A-allele carriers showed a significantly increased TNF protein-expression (p=0.045). **Conclusions:** genomic variants in both TNF and TNF gene are proven to affect *in vivo* TNF- gene-expression. Especially in CAD, an effect of the TNF-PM c.-238G>A and the TNF-PM T60N on gene and protein expression, respectively, could be proven. These results emphasize the importance of the genomic background on the regulation of TNF expression not only in the development of CAD.

Regional absence of mitochondria causing energy depletion in the failing myocardium of the MLP knockout mouse. *B. Bosch van den*¹, *C. Burg van den*¹, *K. Schoonderwoerd*², *P. Lindsey*¹, *H. Scholte*², *I. DeCoo*², *E. Rooij van*¹, *A. Wijngaard van den*³, *H. Rockman*³, *P. Doevendans*⁴, *H. Smeets*¹. 1) Genetics and Cell Biology, University Maastricht, Maastricht, Netherlands; 2) Depts. of Clinical Genetics, Biochemistry and Child Neurology, Erasmus MC Rotterdam, The Netherlands; 3) Dept. of Medicine, Duke University Medical Center, Durham NC; 4) Dept. of Cardiology of the Heart Lung Centre Utrecht (UMCU), The Netherlands.

Defects in myocardial mitochondrial structure and function have been associated with heart failure in humans and animal models. However, the exact role for mitochondrial dysfunction in different stages of cardiac disease remains to be elucidated. Mice lacking the muscle LIM protein (MLP) develop morphological and clinical signs resembling human DCM and heart failure. We tested the hypothesis that defects in the cytoskeleton lead to DCM and heart failure through mitochondrial dysfunction in the MLP mouse model. Our results demonstrate a 35% decrease in overall mitochondrial OXPHOS activity and citrate synthase (CS) activity in the failing hearts. However, activity per amount of CS, a measure for mitochondrial density, was normal, indicating a decreased number of mitochondria in the knockouts. Light and electron microscopy revealed regional absence of mitochondria and a decreased overall mitochondrial size. Mitochondrial and nuclear-encoded transcripts were decreased to 60% of the controls, most likely resulting in decreased mitochondrial biogenesis. Peroxisome proliferator activated receptor gamma co-activator 1 (PGC-1), a key regulator of this process, was decreased to 67% of the controls and corroborates these findings. MtDNA copy number (ratio mtDNA/nuclear DNA) was slightly increased in the knockouts. Our results show that the absence of MLP causes a local loss of mitochondria, leading to energy depletion. This is likely caused by a disturbed interaction between cytoskeleton and mitochondria, which interferes with energy sensing and energy transfer. Recovery of energy depletion by stimulating mitochondrial biogenesis might be a useful therapeutic strategy for improving the energy imbalance in heart failure.

Two novel TNNI3 mutations in restrictive cardiomyopathy. *A. van den Wijngaard¹, D. Merckx¹, C. Marcelis⁴, E. Rubio^{1, 2}, I. de Coo³, C. de Die¹, R. Jongbloed¹, H. Smeets¹.* 1) Clinical Genetics, Academic Hospital Maastricht, Maastricht, Netherlands; 2) Pediatrics, Academic Hospital Maastricht, Maastricht, The Netherlands; 3) Child Neurology, Erasmus MC, Rotterdam, The Netherlands; 4) Clinical Genetics, Radboud Hospital Nijmegen, Nijmegen, The Netherlands.

Troponine I (TNNI3) is a sarcomeric protein expressed in the human ventricular myocardium. The protein is essential for the coupling between the myosin heavy chain globular head and actin during contraction of the cardiac fibers. Occasionally mutations in TNNI3 are found in families with hypertrophic cardiomyopathy (HCM). Recently, also some mutations were reported in patients with restrictive cardiomyopathy. Restrictive cardiomyopathy is a rare cardiomyopathic disorder characterized by impaired ventricular filling with reduced volume, ultimately leading to heart failure. Especially in young children the prognosis is poor compared to adults where the clinical course is more variable. In this study we screened two exons of TNNI3, known to contain the majority of previously identified mutations, in 69 HCM families for mutations by DHPLC. In addition, the complete TNNI3 gene was analyzed by direct sequence analysis in four families with idiopathic restrictive cardiomyopathy. No mutations were identified in the HCM families. However, in two of the four unrelated probands with restrictive cardiomyopathy we found three mutations. In proband 1 we found a novel splice site mutation (IVS7+2delT) and in proband 2 we found a known mutation (R145Q) together with a novel mutation (Arg192Cys). In both families the probands were young girls (age 0.5 and 9 years respectively). The disease manifestation is more severe in proband 1, which might be explained by the difference in the underlying genetic defect. These data indicates that TNNI3 should be analyzed completely when restrictive cardiomyopathy is diagnosed especially in young patients.

RNA interference against a parasitic infection. *V.D.B. Pascoal*¹, *T. Campos-Pereira*¹, *I.G. Maia*², *L.A. Magalhães*³, *E.M. Zanotti-Magalhães*³, *I. Lopes-Cendes*¹. 1) Med.Genet., UNICAMP/FAC. CIENCIAS MED., Campinas, SP, Brazil; 2) Inst. Biocincias, Dept. de Genet., UNESP/Botucatu, SP, Brazil; 3) Dept. de Parasitologia, IB/UNICAMP, Campinas, SP, Brazil.

Approximately two hundred million people are infected with *Schistosoma mansoni* worldwide, demonstrating its high medical importance. *S. mansoni* is unable to synthesize purines *de novo*; therefore, it uses precursors obtained from the host blood, using the essential gene hypoxanthine-guanine phosphoribosyltransferase (HGPRTase), a long-dated potential drug target. RNA interference (RNAi) is a technology that promotes potent and specific gene silencing. It is based on the introduction of small interfering RNAs (siRNAs) that guide target RNA degradation. Its therapeutic use was demonstrated against viruses *in vitro* and *in vivo*. These reports prompted us to investigate the *in vivo* efficiency of this technique against a parasitic disease, by silencing *S. mansoni* encoded HGPRTase gene in infected mice. We chose three target sequences in the *S. mansoni* encoded HGPRTase coding region and produced siRNAs by *in vitro* transcription. Sixty mice were infected with one hundred cercariae each. Seventy days later, animals were either mock-inoculated (PBS buffer) or inoculated with siRNAs (five micrograms diluted in PBS per animal). Six days after the injection, the animals were sacrificed and the number of parasites was counted in the liver and in the porta and mesenteric veins. Overall, the total number of parasites per mouse was reduced by approximately 24% after treatment with siRNAs. The mean number of parasites in the untreated group (n=25) was 39 (median = 40) as compared to a mean of 29 parasites (median = 28) in the treated group (n=23; P = 0.0023, Kruskal-Wallis). It was not possible to determine HGPRTase mRNA reduction in this system; thus we began *in vitro* studies to confirm knock down through RT-PCR. Here we show the successful use of RNAi against *S. mansoni in vivo*. This new approach opens new avenues of research. Our work now expands RNAi therapeutic range to combat parasites, promising a general revolution in disease treatment.

***EFHC1* gene product interacts with Hsp70 and caspase 3.** K. Aguan¹, T. Suzuki¹, A.V. Delgado-Escueta², K. Yamakawa¹. 1) Neuropathology, Brain Science Institute, RIKEN, Wako-shi, saitama, Japan; 2) Center for Integrative Bioscience, Genetics/Genomics Laboratories, Comprehensive Epilepsy Program, David Geffen School of Medicine at UCLA and VA GLAHS-West Los Angeles, Los Angeles, USA.

Juvenile myoclonic epilepsy (JME) is one of the most frequent hereditary grand mal epilepsies. Recently, we have mapped and cloned a JME gene, *EFHC1*, on chromosome 6p12-p11 (Bai et al. Am J Med Genet 113:268-274, 2002; Suzuki et al., in press). *EFHC1* encodes a non-ion channel protein with a single EF-hand motif, interacts with a calcium channel protein, and induces apoptosis in primary culture neurons. As most of the epilepsy genes encode ion channels, conceptually it has been a challenge how *EFHC1* would be involved in epilepsy. In protein array screenings, we identified Hsp70 and caspase 3 as *EFHC1*-interacting proteins. These observations suggests that *EFHC1* is a multifunctional protein that may induce cell death by co-interacting with ion-channel and apoptosis initiating factor, and at the same time possibly regulating the cellular survival response by interacting with Hsp70.

Alternative splicing of *TRIM37*. R. Hämäläinen, T. Joensuu, A-E. Lehesjoki. Folkhälsan Institute of Genetics, Department of Medical Genetics and Neuroscience Center, Biomedicum Helsinki, University of Helsinki, Finland.

The *TRIM37* gene encodes a peroxisomal member of the TRIPartite Motif (TRIM), also known as RING-B-box-coiled-coil (RBCC), protein family. The function of *TRIM37* is yet unknown. *TRIM37* was first identified as the gene underlying Mulibrey nanism, a rare autosomal recessively inherited disorder, characterized by prenatal-onset growth retardation, typical facial features and pericardial constriction with hepatomegaly and an increased risk of developing ovarian tumors and Wilms tumor. To date eleven mutations in *TRIM37* have been associated with Mulibrey nanism. The *TRIM37* gene has 4111 bp of known mRNA transcribed from 24 exons and predicting a protein of 964 amino acids. In northern blot analysis a strong transcript of approximately 4 kb is present in testis, in addition to the 4.5 kb transcript present in all tissues. We have identified and characterized *TRIM37* transcripts using bioinformatics, RT-PCR, real-time quantitative RT-PCR and Northern blotting. *In silico* analysis of *TRIM37* specific EST:s suggests the existence of several alternative *TRIM37* transcripts, that were confirmed by RT-PCR in human testis and lymphoblastoid cell mRNA. One of these novel transcripts corresponds to the 4 kb transcript seen in northern analysis of testis tissue. This transcript has an additional exon 3' to exon 24. It encodes a 964 amino acid protein identical in sequence with the protein encoded by the 24 exon transcript, but an alternative termination codon and 3' untranslated region. In quantitative real time PCR analysis the 24 and 25 exon transcripts have equal expression levels in testis, whereas in other tissues the 24 exon transcript is expressed 6 to 30-fold higher than the 25 exon transcript. Our data suggest that alternative splicing of *TRIM37* is a frequent event, the physiological significance of which remains to be determined.

The interaction between FRG1P and PABPN1 implies a common molecular pathway for the muscular dystrophies FSHD and OPMD. *S.M. van der Maarel¹, S. van Koningsbruggen¹, K.R. Straasheijm¹, H. Dauwerse¹, J.M. Cowan², P.G. Wheeler², H. ter Laak³, B.G. van Engelen⁴, G.W. Padberg⁴, R.R. Frants¹.* 1) Dept Human Genetics, Leiden Univ Medical Ctr, Leiden, Netherlands; 2) Division of Genetics, Department of Pediatrics, Tufts-New England Medical Center, Boston, MA; 3) Department of Pathology, University Medical Center Nijmegen, Nijmegen, Netherlands; 4) Department of Neurology, University Medical Center Nijmegen, Nijmegen, Netherlands.

FRG1 is considered a candidate gene for facioscapulohumeral muscular dystrophy (FSHD) based on its location at chromosome 4qter and the apparent upregulation of its gene product in FSHD muscle. The FRG1 protein (FRG1P) is localized in nucleoli, Cajal bodies and nuclear speckles after stable and transient transfections, which are all structures involved in RNA processing. A putative role of FRG1P in RNA processing is further supported by its nuclear redistribution upon transcription inhibition and its presence in the human spliceosome and in a *C. elegans* cluster of co-regulated proteins involved in ribosomal and mRNA biogenesis. We here show that transient expression of VSV-FRG1 redistributes endogenous PABPN1, the protein mutated in oculopharyngeal muscular dystrophy (OPMD), from the speckles to nucleolar aggregate-like structures. These aggregates are composed of PABPN1, ubiquitin, proteasomes and poly(A) RNA and are resistant to proteinase K treatment, similar to the nuclear aggregates found in OPMD myonuclei. Co-immunoprecipitation experiments with tagged FRG1 and PABPN1 proteins confirmed the interaction. Further evidence for a common molecular pathway in both muscular dystrophies comes from our observation of a FSHD family with possible digenic inheritance involving the FSHD and OPMD locus and from a OPMD patient with a large terminal deletion of 4q including the FSHD locus but without PABPN1 mutation.

Pendred Syndrome and Enlarged Vestibular Aqueduct Syndrome: Identification of six novel mutations in the gene (SLC26A4/PDS). *R. Birkenhäger, A. Aschendorff, T. Klenzner, R. Laszig, J. Schipper.* Department of Otorhinolaryngology and Head and Neck Surgery, University Hospital Freiburg, Killianstrasse 5, D-79106 Freiburg.

Pendred syndrome (PDS), an autosomal-recessive disorder is characterized by sensorineural deafness and goiter [1]. This syndrome is one of the most common forms of syndromic deafness. Hearing loss is prelingual in the majority of the cases, only a subset of patients have a progressive hearing loss later in life. The deafness is associated with temporal bone abnormalities that range from isolated enlargement of the vestibular aqueduct (EVA, LAV) to Mondini dysplasia, a more complex malformation that also includes cochlear hypoplasia. Both, LAV and Mondini dysplasia are easily recognized by computer tomography. PDS is caused by mutations in the SLC26A4 gene, it is located on Chromosome 7q31 and is composed of 21 exons that encode a 780 amino acid protein. This gene product, a transmembrane protein, is called pendrin. Pendrin is expressed in the non-sensory epithelia of the inner ear, renal cortical collecting ducts and proximal tubule of the kidney. Functional studies in *Xenopus laevis* oocytes and Sf9 cells have demonstrated that pendrin is a transporter of iodide and chloride [2]. In this study we analyzed several multiplex families with LAV and hearing loss to distinguish between the Pendred- and LAV-Syndrome. Individual exons and intron transitions of the SLC26A4/PDS gene of patients were PCR amplified. Direct automatic sequencing of variant fragments was performed with the same primers, on an automatic genetic analyser or alternatively after RNA isolation and cDNA synthesis. In our project we describe the mutation analysis of the SLC26A4 gene and identified 13 mutations, 6 of these mutations are novel. In 25 % of our patients we could not identify any mutation. These patients carry potential mutations in regulatory domains such as promotor regions, or the alternative possibility of a distinct locus for a gene of autosomal recessive deafness with enlarged vestibular aqueduct. 1 Everett LA et al. Nat Genet. 1997 Dec;17(4):411-222 Scott DA, et al. Nat Genet. 1999 Apr;21(4):440-3.

Loss of Ubr2 gene function compromises genome stability and double strand break repair mediated by homology recombination. *Y. Ouyang*¹, *Y.T. Kwon*², *A. Varshavsky*³, *Y. Marahrens*¹. 1) Dept Human Genetics, Univ California, Los Angeles, CA; 2) Center for Pharmacogenetics and Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, Pittsburgh, Pennsylvania; 3) 152612, and Division of Biology, California Institute of Technology, Pasadena, California 911253.

Ubr2 is an E3 ubiquitin ligase that functions in the N-end rule pathway for ubiquitin-targeted proteolysis. Previous work showed that deletion of the Ubr2 gene in mouse cells exhibit defects in N-end rule pathway, which leads to *ubr2*^{-/-} female embryonic lethality and male sterility. The male *ubr2*^{-/-} mice have meiotic cells arrested in pachytene stage where homologous chromosomes fail to recombine and this results in massive apoptosis. If Ubr2 mediates homology recombination (HR) in meiosis, we suspect that Ubr2 might also mediate HR in somatic cells. In somatic cells, HR plays a major part in faithful DNA repair when double strand break (DSB) occurs. To test our hypothesis, we use an HR assay developed by Jasins lab which quantifies HR mediated repair and we find that HR mediated repair is much lower in *ubr2*^{-/-} mouse embryonic fibroblasts (MEFs) than in wild type MEFs. Further we find that mitotic spreads made from *ubr2*^{-/-} MEFs have higher frequency of chromosomal breaks. Besides, *ubr2*^{-/-} MEFs display different viability to DNA damaging agents. From all these observations, we conclude that Ubr2 ubiquitin ligase maintains genome integrity and participates in HR mediated double strand break repairs.

The DNA replication profile at the human myotonic dystrophy locus: Determinants of repeat instability. *J.D. Cleary*^{1,2}, *K. Nichol Edamura*¹, *C.E. Pearson*^{1,2}. 1) Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, ON, Canada; 2) Department of Molecular and Medical Genetics, University of Toronto, Ontario, Canada.

The expansion of gene-specific trinucleotide repeats is a form of mutation responsible for at least 15 human diseases, including myotonic dystrophy type 1 (DM1). The cellular processes of DNA replication and/or repair are believed to contribute to repeat instability. Instability of CAG/CTG repeats was mediated by DNA replication in an SV40-primate model system, such that the position of the repeat tract within the advancing replication fork appeared to alter repeat instability. Furthermore, in humans DNA replication may also contribute to repeat instability, as proliferation was required for instability in cultured human DM1 patient-derived fibroblasts. Taken together, these results suggest that the location of replication initiations (origins) and/or their utilization relative to the repeat tract may be an important factor in determining repeat instability. Alternatively, replication fork progression through the repeat tract may contribute to repeat instability.

The replication profile at the DM1 chromosomal locus was determined in DM1 patient cells that were actively undergoing expansions. This analysis suggests that the DM1 locus is located within a region of abundant replication activity, with proximal replication origins located both upstream and downstream of the repeat tract. Within this profile, the repeat tract is located within an inflection point of replication activity. Interestingly, the inflection point also includes both upstream and downstream binding sites for the zinc-finger protein CTCF, implicated as an insulator element at the DM1 locus. These results highlight the possible role of *cis*-elements flanking the repeat tract, including but not limited to CTCF binding sites, in affecting the progress of the replication fork through the DM1 repeat tract and thereby contributing to repeat instability in humans.

Novel alternative transcripts of the oral-facial-digital syndrome type 1 (OFD1) gene in lymphocytes. T.

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Oral-facial-digital syndrome type 1 (OFD1) is an X-linked dominant disease characterized by malformations of the face, oral cavity, and digits. The OFD1 gene has an open reading frame of 3,033 bp over 23 exons, but the function of the OFD1 protein has been unknown yet. Thus far, one alternative transcript of OFD1 using cryptic splice acceptor site in intron 9 were reported in human embryo, retina, and teratocarcinoma other than lymphocyte. We disclose here novel alternative transcripts of OFD1 mRNA extracted from lymphocytes during the process of identification of the OFD1 gene mutation. The proband was the first female child of healthy unrelated parents. She was diagnosed with OFD1 at the age of six months. RT-nested PCR amplification of normal OFD1 mRNA extracted from lymphocytes successfully revealed novel alternative splicing products, including skipping of single exons (10, 11, or 19), two consecutive exons (3 and 4), multiple exons (10 to 12), and the combination of exons (8, 10, 11, and 12). In the index case, novel double deletion mutations were revealed from identification of four novel transcripts, which included three alternative transcripts (skipping of 10, 10 to 11, and 10 to 12). The alternative transcript previously reported was not detected in this analysis. Therefore, alternative splicings of the OFD1 pre-mRNA might be regulated in a tissue-specific manner. The OFD1 protein has five coiled-coil domains and LisH motif. Alternatively spliced exons encode coiled-coil domains or LisH domain, and further study is needed to disclose the role of these novel alternative transcripts.

The Identification of Novel Sarcoglycan-Interacting Proteins. *J. Chen*¹, *M. Skinner*², *A. Wildeman*², *Y-M. Chan*¹. 1) The Henry Hood Research Program, Sigfried and Janet Weis Center for Research, The Geisinger Clinic, Danville, PA 17822; 2) Department of Molecular Biology and Genetics, University of Guelph, Guelph, Ontario, Canada.

Sarcoglycans are a group of single-passed transmembrane proteins predominantly expressed in skeletal and cardiac muscle. They form a specific complex on the muscle membrane via a discrete stepwise assembly pathway and consist of four subunits (alpha, beta, gamma and delta-sarcoglycan). Mutations in sarcoglycans often disrupt the sarcoglycan complex by affecting specific stages in the assembly process and have been shown to cause autosomal-recessive limb-girdle muscular dystrophy (LGMD) as well as dilated cardiomyopathy. Currently, the function of the sarcoglycans or their role in LGMD is not well understood. Using yeast two-hybrid system and database searching, we have identified several novel proteins associated with the sarcoglycans. One of them is a 16kDa subunit of the vacuolar ATPase. Immunohistochemical analysis of muscle biopsy suggests that the 16kDa subunit is predominantly localized in cytoplasm but sarcolemmal staining is also detected. In expression studies, this protein specifically interacts with delta-sarcoglycan but not with the highly homologous gamma-sarcoglycan. In addition, a 16kD deletion mutant lacking the last transmembrane segment was shown to disrupt its interaction with delta-sarcoglycan in overexpression studies. This mutant has previously been shown to disrupt its binding to beta-1-integrin and alter the morphology of L6 myoblasts. The characterization of these proteins and their interactions with the sarcoglycans in muscle should provide important information regarding the function of the sarcoglycans.

MODY locus on 8p23: mutation screening and identification of alternative 5 UTRs in the transcription factor *GATA4* gene. W.M. Mlynarski^{1,2}, M. Borowiec^{1,2}, C. Powers¹, A. Doria^{1,2}. 1) Department of Genetics & Epidemiology, Joslin Diabetes Center, Boston, MA; 2) Harvard Medical School, Boston, MA.

The *GATA4* gene (NM_002052) encodes a member of the GATA family of zinc-finger transcription factors. Mutations in this gene have been associated with cardiac septal defects. The gene is located within the critical region of the maturity-onset diabetes of the young (MODY) locus that we recently described at 8p23 (Kim *et al.*, 2004). In this study, we examined whether sequence differences in this gene underlie this MODY locus. We resequenced the seven known exons and 2 Kb of the promoter region in probands of families showing evidence of linkage at 8p23. This revealed 13 single nucleotide polymorphisms (SNPs), including two missense variations in exon 5 at positions +48754 A/G (Ser377Gly) and +48763 G/A (Val380Met) from the ATG start codon. However, none of these sequence differences segregated with diabetes in our MODY families. Further in silico evaluation of the gene structure showed that there are at least three *GATA4* transcripts with different 5UTRs (ESTs: BI826615, AK097060, BU194442). We confirmed these finding by RT-PCR, RACE and direct sequencing of the cDNAs. Compared to the reference sequence, in which the first exon is located 4.1 Kb upstream from the start codon, two alternative first exons exist, at about 15.9 and 31.0 Kb upstream. Both of these forms have in common a second non-coding exon (271 bp), which is attached directly to the first coding exon of the *GATA4* transcript. These alternative transcripts show a tissue specific pattern of expression. In conclusion, our data exclude mutations in the *GATA4* coding sequence as a cause of MODY. However, our findings of a complex? transcriptional regulation of this gene indicate that the mutation screening must be extended to additional 5 regions before *GATA4* can be excluded as the MODY gene at 8p23.

MyoD-inducible lysosomal sialidase (neu1) is differentially regulated during myogenic differentiation of C2C12 cells. *M. Champigny*¹, *R. Perry*¹, *S.A. Igdoura*^{1, 2}. 1) Department of Biology; 2) Department of Pathology, McMaster University, Hamilton, ON, Canada.

Lysosomal sialidase, encoded by *neu1*, is required for the removal of terminal sialic acid residues from a variety of sialoglycoconjugates. In humans, deficiency of this enzyme results in the inborn error of metabolism Sialidosis, characterized by the accumulation of sialoglycoconjugates within the nervous system and in peripheral organs. A subset of sialidosis patients present with symptoms of profound muscle dysfunction, including progressive muscular atrophy. We have previously shown that the 5 regulatory region of murine *neu1* is typical of skeletal muscle-specific genes due to the presence of several E boxes and its responsiveness to stimulation by muscle regulatory factors (MRFs) such as MyoD. Here we report that sialidase activity is increased by 6-fold during the first 24 hrs of differentiation of C2C12 myoblasts followed by an attenuation to pre-differentiation levels by 48 hrs. We demonstrate that the lysosomal sialidase promoter is highly upregulated by MyoD through a mechanism that is dependent on the MyoD chromatin remodelling domain. We also show that the sialidase promoter is repressed by activated MEK. Inappropriate over-expression of sialidase 48 hrs after the onset of differentiation results in down regulation of myogenin as well as myosin heavy chain expression, and in a halt of the differentiation cascade. This study indicates that lysosomal sialidase is a potent regulator of the early stages of myogenesis.

LMX1B promoter characterization and search for kidney specific regulatory elements. S. Endeke, S. Richter, A. Winterpacht. Inst Human Genetics, Erlangen, Germany.

The Nail-Patella Syndrome (NPS) is a rare autosomal-dominant disorder characterized by dysplastic nails, absent or hypoplastic patellae, dysplasia of the elbows, exostoses of the ilia and, in some cases (~50%), nephropathy. Nephropathy is the most serious aspect of NPS and, in most cases, manifests only by chronic benign proteinuria. In approximately 15% of cases, however, the disease develops towards end-stage renal disease. NPS is caused by heterozygous loss of function mutations in the *LMX1B* gene, encoding a member of the LIM homeodomain protein family. Interestingly, only 30-60% of NPS patients develop nephropathy in spite of identical or similar *LMX1B*-mutations. In addition, incomplete penetrance and a strong intrafamilial variable expressivity has been observed. It is suggested that yet unknown genetic modifiers in the mixed genetic background may influence the renal phenotype of NPS patients. In an attempt to identify modifying factors of *LMX1B* action, we started to characterize the promoter region of the human *LMX1B* gene and to search for kidney specific regulatory regions. Extensive computer based analysis and interspecies sequence comparison using the available genomic sequences of human, mouse, chicken and fish revealed several conserved regions upstream of exon 1 and within intron 2. From EST-data, RT-PCRs and expression studies we conclude that some of these upstream regions most probably correspond to a novel gene adjacent to *LMX1B* in antisense orientation. To further characterize possible regulatory regions, we mapped the transcription start point of *LMX1B* by RACE, primer extension analysis and RPA. Different start sites were mapped, but by comparison between RNA from human kidney and skeletal muscle no kidney specific transcription start site could be identified. In silico analysis of the predicted promoter region using the genomatix software correspond quite well with the experimental data. Interestingly, a comparison of the *LMX1B* promoter with known kidney specific promoters revealed a common framework of transcription factor binding sites. These binding sites and their kidney specificity is currently under detailed investigation.

siRNA knockdown of striated muscle -actinins alters muscle fiber-type differentiation in C2C12 cells. L.A.

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Many congenital myopathies are characterized by breakdown of the sarcomere and aberrant localization of skeletal muscle Z line proteins. The Z lines maintain sarcomeric structure by anchoring actin-containing thin filaments and linking adjacent myofibrils. Two key components of the Z line are slow/cardiac -actinin-2 (*Actn2*) and the fast fiber isoform, -actinin-3 (*Actn3*). To investigate whether these two isoforms differentially affect muscle development and function, we used siRNA knockdown (KD) technology to specifically ablate either *Actn2* or *Actn3* expression in cultured mouse C2C12 myoblasts. A selectable retroviral vector system was used to generate stable C2C12 cell lines that express various short hairpin RNAs under the control of the U6 RNA polymerase III promoter. After testing several alternate siRNAs for each gene, stably transfected *Actn2* KD, *Actn3* KD, and negative control nonsense siRNA-KD cell lines were generated in triplicate. Quantitative rtPCR assays confirmed that both *Actn2* and *Actn3* mRNA levels were specifically reduced to 5% or less of control mRNA levels. Western blot analyses indicated that protein expression was also significantly and specifically reduced. Both *Actn2* and *Actn3* KD cell lines differentiate into contracting myofibers. Interestingly, *Actn2* KD C2C12 cell lines differentiated into particularly large contracting myotubes more rapidly than *Actn3* KD or control cell lines. Furthermore, indirect immunofluorescence and western blot analyses demonstrated decreased levels of slow muscle myosin heavy chain protein in the *Actn2* KD cells. Conversely, *Actn3* KD cells had decreased levels of fast muscle myosin heavy chain. These data suggest that fast and slow muscle fiber development may be impacted by the presence of *Actn2* and *Actn3* transcripts and/or proteins. To further study differences between the *Actn2* and *Actn3* KD cell lines, we are performing gene expression profiling of these KD and control cell lines. This will enable us to analyze expression changes for multiple fiber-type specific genes as well as detect other fundamental differences between *Actn2* and *Actn3* KD cell lines.

Synthesis and processing of mutant JAGGED1 proteins *in vitro*. M. Meunier-Rotival¹, J. Boyer¹, C. Crosnier^{1,2}, C. Driancourt¹, N. Raynaud¹, M. Hadchouel^{1,3}. 1) Cholestases genetiques, INSERM E00-20, Kremlin-Bicetre, France; 2) Vertebrate Dev. Lab., Cancer Res. UK, London, UK; 3) Hopital de Bicetre, France.

Introduction : Heterozygous mutations in JAGGED1 (JAG1), encoding a single-pass transmembrane ligand for Notch receptors, are responsible for Alagille syndrome (AGS). These mutations map to the extracellular and transmembrane domains of JAG1, and 70% of them give rise to a premature termination codon (PTC). The effects of only 3 JAG1 mutations have been studied *in vitro*: in all cases, missense mutations cause an abnormal glycosylation pattern and an abnormal intracellular transport of the defective protein. To gain insight into the possible synthesis and processing of other abnormal JAG1 proteins with missense or PTC mutations, we performed *in vitro* studies in cell culture. **Methods:** Wild-type *JAG1* cDNA or *JAG1* cDNAs with AGS mutations (1 missense, 2 PTC) were cloned in the pcDNA3.1 expression vector and transfected in COS7 cells. Synthesis, processing and localization of JAG1 were studied by western blots and immunohistochemistry. **Results:** Transient transfection experiments showed that wild-type JAG1 protein and JAG1 with a missense mutation located in EGF2 were both synthesized in COS7 cells, and cleaved at the level of the transmembrane domain. In both cases, only the extracellular domain was found in the culture medium. The truncated proteins resulting from PTC in EGF5 or cysteine-rich domain were synthesized, addressed to the membrane and were recovered in the culture medium. **Conclusion:** We have shown that mutant JAG1 proteins can be synthesized *in vitro* and released in the culture medium. These results suggest that mutant proteins could signal outside of the cell (if mutant transcripts escape nonsense mediated mRNA decay) and support a dominant negative effect of mutant JAG1 proteins in the molecular mechanisms of Alagille syndrome.

Cellular homolog of the poxviral p28/N1R RING virulence factor. *T.A. Gray¹, P.J. Fortin¹, R.D. Nicholls²*. 1) Genomics Inst, Wadsworth Ctr, New York State Dept Health, Troy, NY; 2) Ctr for Neurobiology and Behavior, Dept of Psychiatry, University of Pennsylvania, Philadelphia, PA.

Many non-essential poxvirus genes are derived from captured host cDNAs and usually encode products that act in the same host pathways as their cellular homologs. The poxviral p28/N1R virulence factor features a prominent C₃HC₄ RING zinc-finger in its C-terminus. Orthologs of this factor are found in all vertebrate poxviruses, but are mutated in several less virulent vaccinia virus strains. This RING zinc-finger is 44% identical and 60% similar to the RING zinc-finger of the cellular Makorin protein family. Makorin proteins are characterized by multiple C₃H zinc-fingers, the RING zinc-finger, and a distinct, conserved, cysteine-histidine motif that may represent a novel zinc-finger. In addition to the RING zinc-finger, fowlpox and canarypox homologs also encode a Makorin-like C₃H zinc-finger and the distinct cysteine-histidine motif, firmly establishing a homologous link between the cellular and viral genes. Elucidating the function of the cellular Makorin proteins may provide insight into the pro-virulent function of the poxvirus factors. To that end, we are characterizing a mouse *Mkrn1* insertional mutant line to examine the role of *Mkrn1* in mouse development and to evaluate these mice for changes in poxvirus susceptibility. Homozygous *Mkrn1*^{-/-} mice, with less than 0.3% of wildtype *Mkrn1* transcript levels, are born at expected ratios and have no overt developmental phenotype. Importantly, our mice lacked the severe kidney and skeletal dysmorphic features of a recently reported mouse model that were attributed to a partial down regulation of *Mkrn1*. The reported mouse model resulted from a transgenic insertion/deletion near a *Mkrn1* pseudogene (*Mkrn1-p1*), and a paradigm-shifting conclusion was drawn stating that pseudogenes can regulate their source genes. Our mouse line and supporting molecular data clearly refute this unconventional molecular mechanism, and suggest that another mechanism must cause the kidney and bone phenotype observed in the reported mouse model. We are currently evaluating our *Mkrn1* mutant mice for more subtle phenotypes relevant to poxvirus virulence.

Gene duplication at the AZFc region on the human Y chromosome caused by sister chromatid exchange. Y.W. Lin^{1, 2}, P.L. Kuo³, C.C. Hsu⁴, W. Schempp⁵, D.A. Doan Thi⁵, P.H. Yen^{1, 6}. 1) Institute of Biomedical Sciences, Academia Sinica, Taiwan; 2) Graduate Institute of Life Sciences, National Defense Medical Center, Taiwan; 3) Department of Obstetrics and Gynecology, National Cheng Kung University, Taiwan; 4) Department of Obstetrics & Gynecology, China Medical University, Taiwan; 5) Institute of Human Genetics and Anthropology, University of Freiburg, Germany; 6) Department of Pediatrics, Harbor-UCLA Medical Center, Torrance, CA, U.S.A.

The human Y chromosome plays a major role in spermatogenesis. It contains several genes/gene families that are expressed specifically in the testis. About 10% of subfertile men with idiopathic azoospermia or severe oligospermia have microdeletions on their Y chromosome, frequently in the AZFc region. Most men have four *Deleted in Azoospermia (DAZ)* genes within AZFc. These genes contain 1-3 copies of RNA binding motifs and 7-24 copies of DAZ repeats. Recently a polymorphism for a 1.6 Mb deletion at the AZFc region was reported. The so-called gr/gr deletion, present in about 2% of the male population, removes two of the four DAZ genes and is a significant risk factor for spermatogenic failure. One of the possible mechanisms underlying the deletion is nonallelic homologous recombination between sister chromatids, which would generate a reciprocal recombination Y chromosome containing 6 DAZ genes. We designed a novel DNA blot hybridization strategy to analyze the DAZ genes in genomic DNA samples. We found that 5 of the 82 samples we studied had 6 DAZ genes. The presence of 6 DAZ genes in three of the 5 cases was confirmed by interphase FISH analysis. Further studies of the DAZ repeat regions within the DAZ genes identified additional polymorphisms that could have been generated only through sister chromatid exchange (SCE). The results provide strong evidence that SCE in germ cells plays a significant role in the genesis of deletions, duplications and polymorphisms of the human Y chromosome. Our hybridization protocol also serves as a new tool for identifying novel Y chromosome variants and for tracing human migration in the context of Y chromosome evolution.

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Apparent instability in the normal range at the *FMRI* locus: clinically significant or red herring? *D.J. Allingham-Hawkins, T. Ashfield, J. Guo, D. Kennedy, D. Ramadeen, R. Rezaie, M. Shama, M. Skuterud, C. Stanley, J. Steer.*
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We previously reported apparent instability in the normal range at the *FMRI* locus in a series of 9 patients with developmental delay (Tzountzouris *et al.*, 2000, *Genet Test* 4(3):235). We have now detected this apparent instability in 26 patients and/or their parents referred to the laboratory for testing for fragile X syndrome. Of the 26 subjects, 19 (73%) were male and 6 (27%) were female. The majority of subjects were referred because of symptoms of developmental delay (17/26 or 65%) but 7 subjects (27%) were referred because of a family history of developmental delay and 2 subjects (8%) were referred because of apparently unstable alleles in their children. All 19 males were found to have two alleles by PCR but a single band at 2.8 kb by Southern blot indicating the presence of a single X chromosome. The presence of Y chromosome sequences was confirmed by PCR for all male subjects. Of the 6 females, 5 had 3 alleles by PCR while the remaining female had 4 alleles by PCR. All 6 female subjects had a normal female pattern (2.8 and 5.2 Kb) by Southern blot. Other extensive studies in several of the patients including paternity testing, karyotyping, FISH and haplotyping indicated that there was no obvious explanation (non-paternity, sex chromosome mosaicism, chimerism) other than instability for the results. There were two "familial" instances of instability - one in a mother/daughter and one in a father/son. One might envision the transmission of the same unstable allele from mother to daughter but this is not the case in the father to son "transmission" suggesting there may be another mechanism - perhaps autosomal - to explain this instability. These findings represent a dilemma in the clinical lab because the clinical significance, if any, is unclear. A study looking for the presence of unstable alleles in the general population (no developmental delay or family history of such) at the same frequency as seen in our fragile X referrals (approximately 1%) may help to differentiate whether this is a true phenomenon or simply a polymorphism with no clinical significance.

Alterations in the length of the c-terminal alanine tract in Zic2 modulate both DNA binding and transactivation activity. *S. Brown, M. Paraso, L. Brown.* Dept OB/GYN, Columbia Univ, New York, NY.

Alanine tract expansion mutations in transcription factors, including ZIC2, have been shown to cause at least 8 different malformation syndromes, suggesting the possibility of a common pathogenic mechanism. However, the role alanine tracts in determining protein structure and function is unknown. In some cases, alanine tract expansion has resulted in loss of function and in other cases, gain of function. With this in mind, we have constructed cDNA clones in which the normal 15 alanine tract of Zic2 has been expanded to 25, mimicking a recurrent human mutation. In addition, we have created alleles containing an expansion to 35 alanines as well as a 13 alanine deletion. Luciferase assays using a promoter element from the apolipoprotein E gene show that the 25 alanine expansion results in near complete loss of transactivation whereas the shorter alanine tract allele results in increased activity compared to the wild-type protein. In contrast, luciferase assays using the SV-40 promoter show that alanine tract expansion has much less effect on transactivation activity, indicating that the effects of alanine expansion are promoter dependent. Competition experiments indicate that the 25A allele can only partially suppress the activity of the wild type allele even when in 10 fold molar excess, suggesting diminished DNA binding. To test this hypothesis, we have used electrophoretic gel shift mobility assays (EMSA) to determine the relative DNA binding of these alleles. Surprisingly, EMSA, using sequences from the apolipoprotein E gene show that DNA binding is reduced by alanine tract expansion. On the other hand, deletion of the alanine tract results in a five fold increase in DNA binding, a result that is in keeping with its increased transactivation activity. Thus, the Zic2 alanine tract apparently modulates DNA binding as well as transactivation activity. This result opens the possibility that alanine tracts may have a similar role in other transcription factors.

Different Processes of Trinucleotide Repeat Instability Occur in Cultured Fibroblasts of DM1, HD & FRAXA Patients. *R. Lau¹, Z. Yang¹, K. Nichol Edamura¹, D. Chitayat², C.E. Pearson^{1,3}.* 1) Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) University Health Network; 3) Department of Molecular & Medical Genetics, University of Toronto, Toronto, Canada.

Somatic instability of the DMPK CTG tract is high in multiple tissues of Myotonic Dystrophy (DM1) patients. In contrast, the Huntington's disease (HD) CAG tract and the Fragile X (FRAXA) CGG repeat display low levels of instability. Using primary fibroblasts derived from DM1 patients we observed spontaneous and synchronous expansions of the (CTG)₂₁₆ DM1 tract in proliferating but not in quiescent cells. However, the (CAG)₁₈₀ HD tract in proliferating primary fibroblasts of HD patients was stable, and unaffected by induced quiescence. The (CGG)₆₆₂ FRAXA tract in proliferating primary fibroblasts of a FRAXA patient did not show any changes detectable by Southern blotting.

Cells were also treated with agents known to alter DNA synthesis but not directly damage DNA; mimosine blocks the initiation of replication; emetine blocks lagging strand synthesis at replication forks; and the DNA polymerase inhibitor aphidicolin blocks both leading and lagging strands. In DM1 cells, the magnitude of expansions was significantly enhanced by exposure to aphidicolin or emetine. The effects were specific to the expanded disease allele. Mimosine treatment had no effect upon DM1 CTG instability. Treatment of HD cells differed dramatically; mimosine or emetine resulted in small contractions of the expanded HD CAG tract, while aphidicolin had no effect. This tendency towards contraction was not evident in the HD cell line containing (CAG)₄₅, a size more representative of typical HD. Treatment of FRAXA cells also differed; aphidicolin treatment resulted in small contractions of the expanded FRAXA CGG tract, while length alterations could not be detected following either mimosine or emetine treatments.

The different patterns of instability and the differential effects of various replication inhibitors on expanded CTG/CAG tracts of similar lengths in DM1 and HD cells suggests that different processes are regulating the instability at these genetic loci.

Repair of slipped-strand DNAs formed by disease-associated CTG/CAG repeats. *G.B. Panigrahi, R. Lau, M.R. Blondin, C.E. Pearson.* Genetics and Genomic Biology, Hospital for Sick Children, Toronto, Ontario, Canada.

CTG/CAG instability is the cause of 14 diseases including myotonic dystrophy (DM1) and Huntingtons disease (HD). In humans repeat instability can occur in proliferating and quiescent cells. Depending upon the disease, the tissue or developmental stage the instability manifests as either an expansion-bias, deletion-bias or stable maintenance of the repeat tract. The majority of proposed mechanisms for repeat instability involve slipped-strand DNA structures formed by the repeats either in breaks at non-replicating DNA or at replication forks. In both cases, slip-outs of the nicked or continuous strands represent intermediates of expansion or deletion events, respectively. Processing of these intermediates, either independent of and/or in conjunction with DNA replication, may be one of the underlying processes responsible for repeat instability (or stability).

Repeat instability can be perturbed in model organisms that are deficient in various repair processes. However, DM1 and HD patients that display repeat instabilities are not known to be deficient in any repair activity. Thus, repeats from these patients can be expanded, deleted or stably maintained in cells that are repair-proficient. To date, there is no direct experimental evidence of how repair, in a repair-proficient state may contribute to the various kinds of repeat instability.

We established an in vitro assay to determine the fidelity of slipped-DNA processing by human cell extracts that are functional in many DNA metabolic processes. As substrates, we used structurally defined slipped-DNAs containing an excess of CTG or CAG repeats and a single nick. Our results indicate that a variety of repair outcomes can arise. Importantly, the choice of repair path depends critically upon the biophysical features of the slipped-DNAs. The formation of specific DNA intermediates and their distinct repair outcomes may explain the different mutation patterns occurring at each of the various genetic loci or between tissues.

Heterogeneity of Class I insulin VNTR alleles and insulin secretion. *S. LE FUR, C. AUFRAY, C. LE STUNFF, P. BOUGNERES.* Dpt of Pediatric Endocrinology & U561 INSERM, Hôpital Saint-Vincent de Paul, Paris, France.

The polymorphism of the INS VNTR locus is associated with variations of insulin gene transcription and is also a susceptibility locus for Type 1 diabetes (T1D) known as IDDM2. As a group, "short" Class I alleles of the VNTR are associated with increased circulating insulin (Le Stunff, *Nat Genet* 2000, 26: 444-446), and with predisposition to T1D. Since all Class I alleles do not equally predispose to T1D (Stead, *Hum Mol Genet* 2000, 9: 2929-2935), we examined the effect of Class I allele genomic structure on insulin secretion in obese children. Class I VNTR alleles were classified according to the presence of a multi four "A" repeat (3 x 4A) motif in the VNTR part located close to the insulin gene. "A" repeat is ACAGGGGTGTGGGG. Four "A" sequences have been associated in vitro with an increase of insulin gene transcription (Kennedy, *Nat Genet* 1995, 9: 293-298). We compared the values of the insulinogenic index in the different Class I genotypic groups, using the Fisher PLSD test. The insulinogenic index was 107.125 (SD) in obese children with two VNTR alleles encompassing the 3 x 4A motif (N = 32) versus those with only one of such alleles (71.55, p = 0.02, N = 179) or those who do not have this motif (78.53, p = 0.005, N = 256). These data suggest that the functional Class I VNTR alleles may be more heterogeneous than previously thought and may depend on the presence of this 3 x 4A motif.

Searching the target genes for human single-minded 2 (SIM2) transcription factor. *Y. Shimizu*¹, *A. Yamaki*¹, *S. Asai*¹, *J. Kudoh*², *N. Shimizu*². 1) Dept Medical Genetics, Kyorin Univ Sch Health Sci, Hachioji, Tokyo, Japan; 2) (2) Department of Molecular Biology, Keio University School of Medicine, Shinjuku-ku, Tokyo, JAPAN.

Human SIM2 gene, located on chromosome 21q22.2, is a homolog of *Drosophila* single-minded gene which plays a key role in the midline cell lineage of a central nervous system. SIM2 interacts with ARNT2 and these two proteins belong to the basic helix-loop-helix/PAS (Per-Arnt-Sim) transcription factors. SIM2 and ARNT2 form heterodimers but the downstream target genes are not known. To find the target genes of SIM2/ARNT2, we have established the stable cell lines which express continuously SIM2 or SIM2/ARNT2. We made the constructs of pIRES-puro GFP vector, pIRES-puro-SIM2 GFP and pIRES2-hyg-HA ECFP ARNT2, transfected into HeLa cells and selected the transfectants in the presence of puromycin or puromycin/hygromycin. Then, we have performed the differential display analysis using RNA from these four cell lines (parental HeLa, HeLa-mock, HeLa-SIM2 and HeLa-SIM2/ARNT2). After cDNAs were synthesized from RNA, the PCR reactions were done using 5 different downstream primers and 24 upstream primers. We have purified the bands showing different patterns among the cell lines in polyacrylamide gel electrophoresis and cloned into pT7 Blue-3Blunt vector. Sequencing analysis and homology search identified 26 potential target genes of SIM2 and SIM2/ARNT2. Among these holocarboxylase synthetase which can link biotins to histone, was inhibited and hyaluronan synthase 2 was stimulated by overexpression of SIM2 and SIM2/ARNT2. Further analysis of the expressions of these target genes may provide new insight into the molecular function of SIM2 and SIM2/ARNT2 in Down syndrome phenotype.

Identification and characterization of a new serpin-like gene in the 14q serpin cluster. *G. Macintyre, C. Singh, B. Cavilla, D.W. Cox.* Dept. Medical Genetics, University of Alberta, Edmonton, AB, Canada.

Serine protease inhibitors, serpins, are involved in various processes, including coagulation, prevention of tissue degradation, inflammation and tumorigenesis. Human 1-antitrypsin (AAT), a serpin, is synthesized in the liver and transported to the lung, where it is the major inhibitor of neutrophil elastase. AAT deficiency can result in chronic obstructive pulmonary disease and emphysema, but can also affect other tissues and is linked to a number of diseases, including liver disease. 1 antichymotrypsin has also been associated with liver and lung disease. The AAT gene is located at 14q32.1, within a gene cluster containing related serpin genes that have apparently arisen by gene duplication. The cluster includes PI (*SERPINA1*, the AAT gene), a PI pseudogene, and genes encoding alpha1-antichymotrypsin, corticosteroid-binding globulin, protein C inhibitor, kallistatin and *SERPINA10*. Recently, two more serpin-like genes have been identified in the cluster, *OL-64* and *GCET1*. We have re-examined the serpin cluster, in the search for new genes that may be disease related. Our *in silico* analysis revealed at least three additional genes in the cluster, including *OL-64* and *GCET1*, with similarities to the other serpins, suggesting that these encode previously unknown proteins possessing serpin-like activity. EST information and RT-PCR analyses of liver cDNAs indicates that these genes are expressed in liver cells. Other groups have since shown that *GCET1* is expressed in germinal B-cell centres and *OL-64* in visceral adipocyte tissue. The third gene, newly identified in our study, putatively named, *SERPINA14*, is specifically expressed in liver tissue. The tissue-specific gene expression of other cluster members, considered to have predominant expression in the liver, has revealed additional expression elsewhere, including respiratory tissues. The potential role of the 14q cluster serpins in disease needs to be explored.

Expression analysis of netrins-G1 and -G2: Human netrin-G1 isoforms show evidence of differential expression.

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Netrins-G1 and -G2 form a distinct subgroup within the UNC-6/netrin gene family of molecules that have been shown to guide cell growth cone migrations. These recently identified genes differ from classical netrins by three main features: the presence of a glycosyl phosphatidyl-inositol lipid site for membrane anchorage, the generation of multiple isoforms and a non-responsiveness to receptors for classical netrins. In this study we examined the mRNA expression patterns of human netrin-G1 and -G2 and found that whilst both genes show limited non-neuronal expression, there is wide expression in the brain, particularly of netrin-G2. Using a semi quantitative PCR assay, we set out to identify human mRNA isoforms based on the prevalence of variants in the mouse. We identified a total of nine isoforms, three of which were highly expressed in the brain and three isoforms, one of which was highly expressed in the kidney. All variants were derived from the alternative splicing of five exons that code for the unknown domain and three EGF like domains. This data suggests that netrin-G1 isoforms are differentially expressed between the two tissues and that this mechanism may provide further specificity of function. No human isoforms of netrin-G2 were detected in this study. Additionally, we mapped the genomic layout of the human netrin-G1 and -G2 genes and confirmed their chromosomal localization at 1p13.3 and 9q34. In conclusion, our data suggests that the function of netrin-G1 may be fine tuned by the expression of multiple isoforms.

Comparative sequence analysis reveals the presence of bacterial sulfatase operons and a common evolutionary origin between sulfatases and pyrophosphatases. *M. Sardiello*¹, *A. Ballabio*^{1,2}. 1) Telethon Institute of Genetics and Medicine, Naples, Italy; 2) Medical Genetics, Department of Pediatrics, Federico II University, Naples, Italy.

Sulfatases perform important roles in many biological processes, including the turnover and degradation of sulfated compounds. Several human genetic diseases are due to deficiency of specific sulfatase activities. We and others recently identified the gene responsible for Multiple Sulfatase Deficiency (MSD), an intriguing human disease in which all sulfatase activities are simultaneously defective. This gene, named Sulfatase Modifying Factor 1 (SUMF1), is involved in the post-translational modification of a cysteine residue into C-formylglycine at the active site of sulfatases a key step for catalytic activity of sulfatases in most living organisms, with the exception of some bacteria that have sulfatases carrying a serine (instead of a cysteine) that gets modified into formylglycine by a different modifying factor (AslB). An exhaustive search for genes encoding sulfatases and sulfatase modifying factors in all sequenced organisms revealed that: 1) In many instances modifying factor genes are physically associated to sulfatase genes in prokaryotes, indicating the presence of sulfatase operons. SUMF1 bacterial genes flank cys-type sulfatase genes in 70% of the cases, while AslB genes flank both ser-type and cys-type sulfatase genes, strongly suggesting that AslB can modify both types of sulfatases. 2) The domain containing the sulfatase active site shows intra-species sequence convergence, likely due to the evolutionary pressure for the active site to meet precise requirements of the modifying factor in each species. 3) Sulfatases show significant sequence similarities with a subgroup of pyrophosphatases (PPases) and the structures of PPases and sulfatase active sites are similar. Most likely, sulfatases evolved from PPases after the appearance of the post-translational modification. Finally, we completed the catalog of human sulfatases with the identification of four novel genes, leading to a total of 17 genes. These data will be useful for both functional and disease association studies.

***NOS3AS*, the overlapping antisense transcript with endothelial nitric oxide synthase (*NOS3*), encodes a putative mammalian autophagy protein highly expressed in placenta.** *T. Yamada*¹, *A.R. Carson*¹, *G.B. Robb*², *I. Caniggia*³, *T. Yoshimori*⁴, *P.A. Marsden*², *K. Nakabayashi*¹, *S.W. Scherer*¹. 1) Dpt. of Genet., The Hosp. for Sick Children, Toronto, ON; 2) Dpt. of Medicine, Univ. of Toronto, Toronto, ON; 3) Samuel Lunenfeld Res. Inst, Mt. Sinai Hosp., Toronto, ON; 4) Dpt. of Cell Genet, Nat. Inst. for Genet., Japan.

The human *NOS3* gene mapped on 7q36 encodes a nitric oxide synthase. Some DNA polymorphisms in the gene are reported to be associated with cardiovascular diseases and preeclampsia. We have identified *NOS3AS* as an overlapping antisense transcript with *NOS3*, and have shown that it participates in the post-transcriptional regulation of *NOS3*. Further analyses revealed that *NOS3AS* encodes a 924 amino-acid protein that contains 25% sequence identity with the yeast autophagy gene 9 (*APG9/ATG9*). Autophagy is an intracellular degradation process highly conserved from yeast to higher eukaryotes. Yeast *APG9* encodes a transmembrane domain-containing protein shown to be indispensable for autophagic vesicle formation. Sequence analysis identified two *APG9*-like genes in the human genome: *APG9L1* on 2q35 and *APG9L2* (*NOS3AS*) on 7q36. Northern and RT-PCR analyses revealed that *APG9L1* is ubiquitously expressed, while *APG9L2* is expressed in a tissue-specific manner (pituitary gland and placenta). Phylogenetic analysis for *APG9*-like genes from multiple species revealed that chicken and lower eukaryotic species have only one *APG9*-like gene, which is closer to *APG9L1* than *L2*. Moreover, species higher than chicken have two *APG9*-like genes (*L1* and *L2*), indicating that *APG9L2* is a mammalian-specific gene. However, subcellular localizations by the transient expression of the GFP-tagged protein in COS7 cells determined *APG9L1* and *APG9L2*s distribution to be indistinguishable. Taken together, our data suggest that both *APG9L1* and *APG9L2* are likely to be functionally orthologous to yeast *APG9*, but *APG9L2* may be critical for autophagy in mammalian specific developmental events. Experiments are underway to determine if *APG9L1/L2* are functional in the mammalian autophagy pathway and to examine the possible roles of *APG9L2* in placental development and disease.

Isolation and characterization of human TRUB1 encoding the cytoplasmic and mitochondrial pseudouridine-55 synthase. *J.L. Peters*^{1,2}, *Q. Yan*², *M.X. Guan*^{1,2}. 1) Graduate program of Molecular and Developmental Biology, Cincinnati Children's Hospital, University of Cincinnati, Cincinnati, OH; 2) Division of Human Genetics, Cincinnati Children's Hospital, Cincinnati, OH.

Modified nucleotides of cytoplasmic and mitochondrial tRNA are crucial for tRNA structure and function. This posttranscriptional modification of tRNA is a complicated process catalyzed by enzyme complexes. In particular, bacterial Trub protein catalyzes the formation of pseudouridine (Ψ) at the conventional position 55 in tRNA, a modification highly conserved among all tRNA. The A8344G mutation in corresponding position of human mitochondrial tRNA^{Lys} is associated with MERRF syndrome, a classic mitochondrial disorder. Here, we have cloned and characterized the human counterpart TRUB1. TRUB1 is comprised of 8 exons on chromosome 10 spanning roughly 40kb. The gene encodes a predicted protein of 37,252 daltons. This protein is highly conserved from bacteria and yeast to human, particularly in the central activity domain. Previous studies showed that the TRUB1 gene is ubiquitously expressed across 12 different tissues. Immunofluorescence analysis of human 143B cells revealed that Trub1 co-localized, although not exclusively, with the mitochondrial protein COX1. This result suggests that Trub1 localizes in mitochondria and the cytoplasm. Thus, we propose that Trub1 catalyzes the -55 modification in both cytoplasmic and mitochondrial tRNA and therefore plays a role in modulating the phenotypic manifestation of disease-associated mitochondrial tRNA mutations.

Efficient Transgenesis and Sustained Muscle-Specific Expression of a *Myogenin-EGFP Tol2* Transposon

Construct in Zebrafish. A. Rashid¹, M.J. Ng¹, J. Ben², F.S.H. Cheah², A. Koga³, E.W. Jabs⁴, C.G.L. Lee⁵, C.H. Phang¹, S.S. Chong^{2,4}. 1) Life Sciences & Chemical Technology, Ngee Ann Polytechnic, SINGAPORE; 2) Pediatrics, Ob/Gyn, Lab Medicine, Nat'l Univ Singapore & Hospital, SINGAPORE; 3) Biological Sciences, Nagoya Univ Graduate School of Science, JAPAN; 4) Pediatrics, Institute of Genetic Medicine, Johns Hopkins Medicine, USA; 5) Biochemistry, Nat'l Univ Singapore, SINGAPORE.

Molecularly reconstructed and naturally occurring vertebrate transposons have numerous potential scientific and clinical applications, including insertional mutagenesis, gene trapping, gene transfer, and gene therapy. *Tol2* is a vertebrate transposon occurring naturally in the medaka fish *Oryzias latipes* that encodes its own fully functional transposase. We wished to evaluate the application of *Tol2*-mediated transgenesis to the analysis of gene expression in the living zebrafish. An enhanced green fluorescent protein (*EGFP*) reporter gene driven by a 2.5kb *myogenin* (*myog/myf4*) promoter was cloned into a *Tol2* transposon vector. The recombinant clone was co-injected with *Tol2* transposase mRNA into fertilized zebrafish embryos. EGFP expression was monitored using a fluorescent stereomicroscope. Muscle-specific expression of EGFP was observed as early as 24 hpf in all microinjected embryos. After 13 weeks (3 months), all surviving fish continued to exhibit a mosaic pattern of EGFP expression in the skeletal muscles of the trunk. Of these, 35% showed strong EGFP fluorescence, 29% had moderate EGFP expression, while the remaining 35% showed only isolated patches of EGFP expression. After more than 4 months, 14 of 15 remaining fish continued to express EGFP in a tissue specific manner, EGFP being undetectable in only one of the previously low expressing fish. In marked contrast, EGFP expression in fish injected with a non-transposon plasmid construct carrying the same expression cassette was transient and declined rapidly, with a majority of fish losing expression by 5 days. Outcrossings of founder mosaic transgenic fish will be performed to determine the degree of germline integration and to analyze EGFP expression patterns in constitutive transgenic F1 progeny.

Lgl2 regulates nuclear import of the glucocorticoid receptor and downstream effects on cytokine synthesis in airway epithelial cells. *F. Kaplan*^{1,2,3}, *J. Lan*², *R. Hache*⁴, *T. Tao*^{1,2,5}. 1) Dept of Human Genet., McGill Univ., Montreal, QC, CA; 2) Montreal Child. Hosp. Res. Inst, Montreal, QC, CA; 3) Dept of Pediatr., McGill Univ., QC, CA; 4) Ottawa Health Res. Inst and Dept of Med. and Biochem., Univ. of Ottawa, ON, CA; 5) Key Laboratory of the Ministry of Education for Cell Biology, Xiamen University, Xiamen, China.

Anti-inflammatory effects of glucocorticoids (GC) are critical to treatment of airway inflammation in such common disorders as asthma and COPD. There is a considerable variation in responsiveness to GC and prolonged exposure to GC can result in GC resistance. We reported the cloning of LGL2, a GC inducible gene in fetal rat lung cell culture. Comparison with sequences in the genome database identified lgl2 as a member of the importin- family of nuclear import receptors. We described the functional characterization of lgl2 as an importin- protein and demonstrated developmental regulation of its nucleocytoplasmic shuttling in fetal lung. We now report on the identification of the GC receptor (GR) as a primary cargo substrate for lgl2. To our knowledge this is the first identification of a nuclear transport carrier for a specific hormone receptor. We show that C-terminal lgl2 (which includes the ligand-binding domain) competitively inhibits nuclear entry of GR in HeLa cells. To investigate the potential role of lgl2 in modulating GC-GR regulated events in the lung, we studied lgl2 regulated GR transport in airway epithelial cells. The pro-inflammatory cytokine TNF- potently stimulates airway epithelial cells to produce cellular inflammatory cytokines including IL-8, an effect inhibited by GC. We show that an LGL2 siRNA that inhibits lgl2 synthesis in A549 lung epithelial cells prevented nuclear translocation of GR. Moreover, silencing of lgl2 in siRNA treated cells abrogated the ability of cortisol to inhibit synthesis and secretion of IL-8 following stimulation with TNF-. Our findings support a role for lgl2 in regulation of GC anti-inflammatory effects mediated by GR. We speculate that variation in both cellular levels of lgl2 and intracellular lgl2 shuttling rates may contribute to GC resistance.

Localization of regulatory elements of the human LMX1B gene with transgenic mice. *L. Liu, J.A. Dunston, C. Tran, I. McIntosh.* Inst Genetic Medicine, Johns Hopkins University, Baltimore, MD.

Nail Patella Syndrome (NPS) is an autosomal dominant, pleiotropic condition affecting the nails, patellae, skeletal system, kidneys, and eyes. NPS is caused by mutations in the LIM homeodomain transcription factor LMX1B. To identify sequences responsible for the spatio-temporal expression of LMX1B gene, transgenic mouse lines were established using two overlapping bacterial artificial chromosomes (BACs), 123K19 and 265A2, containing varying distances upstream and downstream of the gene. Each BAC was digested with *NruI* or *SalI* (named as 19S, 19N, 2N, respectively), and then injected into fertilized mouse eggs. F1 transgenic embryos were analyzed by *in situ* hybridization mid-gestation. Consistent with the published data, the expression of mouse *lmx1b* gene was observed in the limb, spinal cord, mammary gland and midbrain-hindbrain junction. The segments defined by the BAC clones revealed expression of the transgenic LMX1B gene in distinct anatomical locations comparable to mouse *lmx1b* except for the limb. The 19N transgenic embryos showed gene expression in the dorsal part of both the forelimb and hindlimb, while the expression of transgene in 19S and 2N was restricted to the shoulder and pelvic girdles. This preliminary data suggests that regulatory elements 25-65kb upstream of the LMX1B gene regulate its expression in dorsal limb. Local alignments of genomic sequences were determined for the LMX1B genes from human, mouse, rat and chick with the MultiPipMaker or AdvancePipMaker program. Within this 40-kb region upstream of LMX1B gene, 5 regions longer than 100 bp with greater than 80% identity were found to be conserved between mammals. Only two of them were also conserved in chick and were analyzed for a common framework of transcription factors.

The TRPS1 transcription factor is associated with promyelocytic leukemia (PML) nuclear bodies through the interaction with the Topoisomerase I binding RS protein (TOPORS). *H.-J. Lüdecke¹, S. Weger², B. Horsthemke¹, F.J. Kaiser¹.* 1) Inst. for Human Genetics, University Clinic, Essen, Germany; 2) Dept. of Virology, Free University, Berlin, Germany.

The TRPS1 gene on human chromosome 8q24.1, which is mutated in the tricho-rhino-phalangeal syndrome, encodes a repressor of GATA mediated transcription. To identify co-factors of TRPS1, we performed a yeast two-hybrid screen with the C-terminal 647 amino acids (aa) of TRPS1 as a bait. Among 23 yeast clones obtained, two encode aa 930-1006 of the 1045 aa topoisomerase I binding RS protein (TOPORS). Using a yeast *in vivo* -galactosidase assay, we narrowed down the TOPORS-binding region of TRPS1 to the C-terminal 100 aa (1181-1281). We verified the interaction of endogenous and ectopically expressed TRPS1 and TOPORS in T47D and COS-7 cells by immunochemical precipitation experiments. TOPORS also interacts with Topoisomerase I and the p53 tumor suppressor, and it has been suspected that it functions as a negative regulator of cell growth and possibly as a tumor suppressor itself (Saleem et al., 2004, *Oncogene*, in press). TOPORS interacts with SUMO-1 and the SUMO-1 conjugating E2 enzyme UBC9 and is localized in promyelocytic leukemia (PML) nuclear bodies, which play a role in a variety of transcriptional regulation processes. Weger et al. (2003, *Exp Cell Res* 290, 13-27) have speculated that TOPORS might function as a SUMO-1 E3 ligase. Interestingly, we found that in human primary fibroblasts TRPS1 is almost completely SUMOylated. The endogenous TRPS1 is located in dot-like nuclear structures, some of which also contain PML protein. Whereas ectopically expressed TRPS1 is homogeneously distributed throughout the nucleus of COS-7 cells, it is only found in the dot-like structures when co-expressed with TOPORS. This indicates that TOPORS is involved in the TRPS1-PML association. In a luciferase reporter assay, we could exclude a direct effect of TOPORS on the repression function of TRPS1. We speculate that TOPORS acts as a SUMO-1 E3 ligase on TRPS1 in PML nuclear bodies and thereby modulates the function of TRPS1. (Supported by the Deutsche Forschungsgemeinschaft).

Wnt-4 inhibits the canonical Wnt pathway by redirecting β -catenin to the cytoplasmic membrane. *P. Bernard, A. Lacombe, A. Fleming, E. Vilain.* David Geffen School of Medicine at UCLA, Departments of Human Genetics, Pediatrics and Urology, Los Angeles, CA.

Wnt-4 is a signaling molecule essential for kidney, adrenal and gonadal development. Wnt-4 deletion results in the masculinization of XX mouse pups, and we have previously shown that overexpression of Wnt-4 dramatically reduces testosterone levels in XY mice, suggesting that Wnt-4 acts as an anti-male factor. We have investigated the molecular mechanisms of action of Wnt-4 and shown that it utilizes a novel signaling pathway. Using transient transfections, we have shown that Wnt-4 inhibits TCF and SF1 responsive genes. Specifically, Wnt-4 disrupts the synergistic interaction between SF1 and β -catenin on the promoter of target genes such as the Steroidogenic Acute Regulatory Protein (StAR). However, instead of promoting β -catenin degradation as it has been previously described for inhibitory Wnt genes, Wnt-4 stabilizes β -catenin. In addition, β -catenin accumulates at the cytoplasmic membrane as shown by indirect immunofluorescence assay on cells stably expressing Wnt-4. We confirmed this finding by co-immunoprecipitation with E-cadherin, a membrane-bound protein that binds β -catenin. In the presence of Wnt-4, larger amounts of β -catenin co-precipitate with E-cadherin compared to control. Increased interaction between β -catenin and E-cadherin also promotes E-cadherin-mediated cell-cell adhesion, ultimately leading to decreased cell migration. The latter observation is consistent with previous observations that the lack of Wnt-4 correlates with an increase of steroidogenic cell precursors migrating into the female gonad. Altogether these results demonstrate that Wnt-4 acts through a novel signaling pathway that prevents β -catenin action in the nucleus by redirecting it to the cytoplasmic membrane.

Interspersed 3 AGG interruption in the CGG repeat sequence in the FMR1 gene in mentally retarded patients of unspecified cause. *P.M.K. Poon¹, K.Y.C. Lai², Q.L. Chen², C.K. Wong², C.P. Pang³.* 1) Chemical Pathology, Chinese Univ Hong Kong, Hong Kong, China; 2) Psychiatry, Chinese Univ Hong Kong, Hong Kong, China; 3) Ophthalmology & Visual Sciences, Chinese Univ Hong Kong, Hong Kong, China.

We have shown that the CGG repeat pattern of the FMR1 gene is different in mentally retarded patients from the normal population. Haplotypes involving polymorphic markers flanking the FMR1 gene, CGG-FRAXAC2 and FRAXAC2-DXS548, also distributed differently. Since 3 AGG interruption in the FMR1 CGG repeat sequence affects stability of CGG stability, we investigated the link between AGG interspersion pattern and mental status. We determined the FMR1 CGG substructure in 104 normal healthy male subjects and 232 unrelated male patients with mental retardation of unspecified cause. All the study subjects had less than 53 CGG repeats and therefore did not have fragile X syndrome of mental retardation. There is a significant difference ($p < 0.006$) in the AGG interspersion pattern between the normal controls and the mentally retarded patients. Although the majority of both groups of subjects have 2 interspersed AGG, the mentally retarded patients have proportionally less 2 and 3 AGG but more 0 and 1 AGG than the normal controls. They also have a lower frequency of (CGG)10AGG(CGG)10AGG(CGG)9 but a higher frequency of (CGG)10AGG(CGG)19 alleles. Their pure CGG repeat at the 3 position is longer and their CGG internal configuration is less variable. Our results show that the AGG interspersion pattern is associated with mental retardation prior to or independent of the mutational events leading to the development of FRAXA mental retardation.

Functional Genomics of the Human Catecholamine Storage Vesicle Protein Chromogranin B (CHGB). *R.M.*

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Chromogranin B (CHGB) is a member of the chromogranin-secretogranin protein family, comprised of acidic, soluble proteins that are stored in secretory granules with hormones, transmitters, and neuropeptides throughout the endocrine and nervous systems. Interest in CHGB has increased as recent studies suggest that CHGB plays a critical role in intracellular catecholamine compartmentalization and release. To improve the understanding of mechanisms of catecholamine release, and the role that dysfunction may have on development of complex human diseases, we undertook systematic variant discovery by resequencing this locus in 160 ethnically diverse human subjects. We resequenced all 5 exons and adjacent intron regions, ~1 kb upstream of the 5 promoter, 2 conserved promoter regions up to ~4 kb upstream, and 1 intronic conserved regions. A total of 52 SNPs and a single base (C) insertion/deletion were identified. Of these, 19 were common (minor allele >5%) in our sample. Remarkably, 10 SNPs were identified in exon 4, 9 of which encoded amino acid substitutions. A subset of 11 of the identified CHGB SNPs was genotyped in additional phenotyped subjects. 9 of the 11 SNPs predicted CHGB expression in vivo (plasma peptide fragment CgB439-451 level). In 150 twin pairs, 3 of the 11 SNPs [Glu348Glu ($p=0.014$), and His397Arg ($p=0.032$) in exon 4, and C13612A ($p=0.035$) in intron 4] predicted heritable, stress-induced blood pressure increments. In a male population sample with extreme blood pressures (300 high/300 low), systolic blood pressure was elevated in association with the C allele of the T10501C SNP ($F=3.52$, $p=0.024$). We conclude that common allelic variation at the CHGB locus has functional consequences for expression of the gene in vivo, as well as for stress susceptibility of blood pressure variation, and finally for development of hypertension.

Studying RNA instability mutations via a novel model using a BAC clone. A. Eeds¹, R. Wade-Martins³, D. Mortlock¹, D. Piston¹, L. Hall², A. Putnam², T. Nalls², M. Summar^{1,2}, *The Urea Cycle Disorders Consortium*. 1) Center for Human Genetic Research, Dept Molec Physiology, Vanderbilt Univ, Nashville, TN; 2) Dept Pediatrics/Div Medical Genetics, Vanderbilt Univ, Nashville, TN; 3) The Wellcome Trust Centre for Human Genetics, University of Oxford, UK.

Carbamyl phosphate synthetase I (CPSI) is the liver specific enzyme that catalyzes the rate-determining step of the urea cycle. We have found mutations in patients with CPSI Deficiency that may cause RNA instability. We hypothesize that these mutations result in the instability of that RNA message due to splicing defects and premature termination codon formation leading to nonsense-mediated decay and that these mutations can be studied using a BAC clone. Because CPSI is liver specific, patient fibroblast cell lines cannot be used for RNA assays. Also, these mutations are spread throughout the gene, which is composed of 38 exons that span over 120 kb of genomic sequence. Therefore, our focus is to find a way to re-create and test each of these mutations using one construct that can be efficiently manipulated. A BAC clone containing the CPSI genomic sequence was modified to contain a gene for GFP to assay for transfection efficiency, hygromycin resistance for selection in eukaryotic cells, and EBNA-1, a viral gene to aid in episomal retention of the BAC following transfection in eukaryotic cells. We also engineered CPSI to be driven by the CMV promoter for more ubiquitous expression. Putative RNA instability mutations will be rapidly engineered into this construct using homologous recombination with oligos that contain the mutation in question. Following transfection of the BAC construct into eukaryotic cell cultures, the resulting RNA species can be assayed to determine the precise mechanism of the mutation using conventional RNA assays. This model system will allow rapid determination of the mechanisms that the mutations responsible for CPSID employ to cause RNA instability. We anticipate this model system will be widely applicable for studying heterogeneous mutations responsible for genetic disorders as well as for determining if a mutation has an effect on RNA when there is no patient source available.

Analyses of splicing patterns and variations of transcription start sites using human full-length cDNAs constructed by oligo-capping method. *T. Isogai¹, A. Wakamatsu¹, K. Kimura², J. Yamamoto¹, J. Uechi², T. Nishikawa^{1,2}, S. Sugano³, N. Nomura⁴.* 1) Reverse Proteomics Res Inst, Kisarazu, Chiba, Japan; 2) Central Research Laboratory, Hitachi Ltd., Tokyo, Japan; 3) Department of Medical Genome Sciences, Graduate school of Frontier Sciences, University of Tokyo, Tokyo, Japan; 4) Biological Information Research Center, National Institute of Advanced Industrial Science and Technology, Tokyo, Japan.

Full-length cDNA clones are important materials for experimental elucidation of gene function. About 30 thousand of human full-length cDNAs were sequenced by our NEDO human cDNA sequencing project, and were deposited to DDBJ/GenBank/EMBL. From the analysis of human genome sequences, the number of human genes is predicted to be approximately 30K-40K. And the number of mRNA transcribed from the human genome is predicted to be approximately 80K-110K. From these results, a lot of variant cDNAs including splicing and transcription start site variations could be obtained. In order to search splicing variant cDNA efficiently, 29,789 full-length cDNAs of our project and 42,821 cDNAs of other cDNA projects and RefSeq eliminated same sequences as cDNA projects were mapped to 27,803 loci of human genome. For this we found that 12,002 loci consisted of two or more splicing patterns had 41,021 of splicing patterns. Then the variations of the splicing patterns based on ORFs were analyzed. We are analyzing and cloning of variant cDNAs including splicing and transcription start site variations from about 1.4 million cDNAs of about 100 kinds of cDNA libraries consist of human tissues and cells constructed by oligo-capping method. About 500 bp of all 5-end of the cDNAs were obtained by our NEDO human cDNA sequencing project. The full-length rate of 5-end of those was about 90%. We mapped those to human genome for cloning of variant cDNAs based on ORF variations, and then obtained not only splicing variant cDNAs, but transcription start site variations. Some of them had tissue specific expression profiles. This work was supported by a grant from New Energy and Industrial Technology Developmental Organization (NEDO) project of the Ministry of Economy, Trade and Industry of Japan.

DNA Palindromes: The role of DNA repair functions. *J.H. Appleby*^{1,2}, *S.M. Lewis*^{1,2}. 1) Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, Canada; 2) Genetics and Genomic Biology, Hospital for Sick Children, Toronto, Ontario, Canada.

DNA palindromes consist of pairs of inversely repeated sequences having central symmetry that are able to form intra-strand hairpin and cruciform structures. The formation of these structures poses a special threat to the genome as they can block replication and are postulated to lead to gross chromosomal rearrangements such as deletions, translocations, gene amplification and repeat expansion. How palindromes mediate such rearrangement is undetermined. Based upon a murine model, a unique mechanism for dealing with palindromes has been proposed, termed the Center-Break Model. Extrusion of a cruciform occurs from a perfect palindrome, and each of the hairpin tips are nicked, creating a double strand break at the center of symmetry. This break is then repaired by the NHEJ pathway, often resulting in deletion of sequence that creates a central asymmetry.

Two complementary assays are being used in a candidate gene approach to determine which DNA repair functions are involved in palindrome processing. The first assay uses the Line 78 mouse that bears a perfect 15.4kb palindrome on chromosome 17 and in a wild-type background rearranges at a rate of 0.5% per generation. Rates of rearrangements for this chromosomally located palindrome are being determined by crossing the Line 78 mouse with mice deficient for the candidate gene. The Werners syndrome protein, DNA-Pkcs, Ku70, and Artemis are currently being tested for roles in palindrome processing. The second transient assay uses a 15.6kb palindromic circular dimer that is transfected into Abelson cell lines deficient for the candidate gene. The dimeric molecule undergoes rearrangement consistent with the center-break model, and is recovered as a monomer. The number and structure of recovered monomers reveals any deviation in palindrome processing from wild-type.

Sense-antisense RNA transcription and methylation of D4Z4 repeat array in Facioscapulohumeral muscular dystrophy. *L. Felicetti*^{1,2}, *A. Cellini*¹, *M. Rossi*¹, *P. Venditti*¹, *G. Galluzzi*², *L. Colantoni*², *R. Frusciante*¹, *B. Merico*¹, *P. Tonali*¹, *E. Ricci*¹. 1) Institute of Neurology, Catholic University, Rome, Italy; 2) UILDM, Rome.

Linkage and physical mapping strategies have localized the FSHD gene to the subtelomeric region of chromosome 4q, a polymorphic locus containing an array of KpnI repeat units (D4Z4). A decrease in the number of these repeats below the threshold of 10 units is associated with the disease. Despite the presence of an open reading frame containing two homeodomains and a putative promoter region, no protein coding transcripts derived from the D4Z4 locus have been so far identified in vivo (Hewitt et al, 1994). To further investigate RNA transcription from KpnI repeats in FSHD, we searched for D4Z4-derived RNA species that are not limited to the open reading frame sequences present in the region. Using a RNase protection assay and Northern blot analysis with probes positioned across the KpnI sequence, we show that transcription occurs on both DNA strands in muscle biopsies derived from FSHD patients, giving rise to RNAs that may play an essential role in the chain of events leading to the disease. Since we do not find such transcription in muscles obtained from normal subjects, it is conceivable that it represents an inappropriate activity of the KpnI repeat units present on the contracted D4Z4 locus. Consistent with a transcriptional up-regulation of the contracted D4Z4 array, a marked hypomethylation of this region was recently found on both alleles in FSHD patients (van Overveld et al, 2003). Using bisulfite genomic sequencing technique we have analysed the methylation status of two regions within the KpnI units at 4q35. While the first region analysed, showed a marked hypomethylation on both 4q alleles in FSHD patients, as previously described, the second region, showed the same methylation status on 4q alleles in healthy and affected subjects. According to these results the methylation status is not uniformly spread on the entire KpnI unit, underscoring the complexity of the locus where specific subregions could play different roles in the molecular mechanism of the disease.

Identification of a CYP2E1 gene duplication in African American and Caucasian populations using TaqMan Real-Time PCR. *L.Y. Wong, M. Lin, S.-N. Liew, J. Stevens, K.J. Livak, K.D. Lazaruk.* Applied Biosystems, Foster City, CA.

CYP2E1 is a cytochrome P450 enzyme that catalyzes the bioactivation of environmental carcinogens such as N-nitrosamines. A wide range in CYP2E1 expression and activity between individuals has been previously reported. Interindividual variation is thought to be partly influenced by genetic factors. However, no clear large-scale associations between genetic polymorphisms and phenotypes have been established. In this study, we determined that individuals may carry a duplication of the CYP2E1 gene. TaqMan allelic discrimination assays were used to genotype single nucleotide polymorphisms (SNPs) at positions -352A>G, -333T>A, -71G>T, and 4768G>A of the CYP2E1 gene in African Americans and Caucasians. Atypical results were observed for 7 out of 90 individuals when signal intensities from the two allele-specific fluorogenic probes were graphed on a plot. TaqMan real-time quantitative PCR assays were used to determine the dosage of the CYP2E1 5 regulatory region, exon 4, intron 6, and exon 9. The results indicated that the atypical individuals carried 3 copies of each CYP2E1 region assayed while all other individuals carried 2 copies. Dosage data from a CEPH family in which the father carried the CYP2E1 duplication demonstrated that the duplication was inheritable and stable. The prevalences of the duplication in African Americans and Caucasians were estimated at 5% and 11%, respectively. These findings may partially account for interindividual variation in CYP2E1 expression and activity. Further characterization of the duplication will be required to determine whether the duplication is an active gene.

SNP Haplotype at the *MDR1* Promoter affect its activity under ultraviolet irradiation stress. C.G. Lee^{1,2}, S.M. Ngoi². 1) Department of Biochemistry, National University of Singapore, SINGAPORE; 2) Div Medical Sci, National Cancer Ctr, SINGAPORE.

The *MDR1* multidrug transporter plays an important role in shielding the individual against xenobiotic toxicity. Single nucleotide polymorphisms (SNPs) and SNP haplotypes within the *MDR1* gene have been shown to account for variability in *MDR1* expression and function, drug response as well as disease susceptibility. However, the effect of SNPs on *MDR1* promoter activity is not well characterized. Through sequencing of 1.5 kb of sequences at the *MDR1* promoter region in 70 individuals of Chinese ethnic origin, we identified only three SNPs, intron-1 -41A>G, exon1 -145C>G and exon1 -129T>C. These three SNPs have been previously reported. SNPs intron-1 -41A>G and exon1 -145C>G reside in the predicted NF-Y and ETS binding sites, respectively. These SNPs were genotyped in approximately 96 individuals from each of five ethnic populations, Chinese, Malays, Indians, Caucasians and African Americans. All three SNPs were found to occur at frequencies of less than 10% in all populations with SNP exon1 -145C>G being Chinese-specific and SNP intron-1 -41A>G being monomorphic in Indians and of very low frequencies in the Caucasians and African Americans (0.5% minor allele frequency). Of 8 possible haplotypes, 5 haplotypes were observed in the Chinese, 4 in the Malays, 3 in the Caucasians and African American and 2 in the Indians. *In vitro* promoter assays revealed that none of the observed haplotypes significantly affected *MDR1* promoter activity in KB3-1 or N2a cell-lines. However, one haplotype which was not observed in any of the ethnic group examined affected promoter activity significantly under ultraviolet (UV) irradiation stress in KB3-1 but not N2a cell-lines. It is possible that this haplotype occur at a frequency that is lower than what was examined. In summary, our results suggest that SNP haplotypes at the *MDR1* promoter may influence its activity in specific cell-types and/or under specific external stress.

Toward the Evaluation of Function in Genetic Variability: Characterizing Human SNP Frequencies and Establishing BAC-Transgenic Mice Carrying the Human CYP1A1_CYP1A2 Locus. Z. Jiang¹, T.P. Dalton¹, L. Jin¹, B. Wang¹, Y. Tsuneoka², H.G. Shertzer¹, R. DeKa¹, D.W. Nebert¹. 1) Dept Environmental Health, Univ Cincinnati, Cincinnati, OH; 2) Yokohama Hospital, Yokohama Midoriku Terayamachou, Japan.

Inter-individual differences in human CYP1A1 and CYP1A2 expression are believed to be associated with variability in risk toward various types of environmental toxicity and cancer caused by polycyclic aromatic hydrocarbons and aromatic amines present in combustion products. These two genes are oriented head-to-head on human chromosome 15; the 23.3-kb spacer region might contain distinct regulatory regions for CYP1A1 and distinct regulatory regions for CYP1A2, or regulatory regions for the two genes might overlap one another. From the five major geographically isolated human subgroups, we performed SNP-discovery in 24 unrelated subjects by DNA resequencing of both genes (all exons and all introns) plus some 3 flanking sequences and the entire spacer region (39.6 kb total); 85 SNPs were found, 49 of which were not currently in the NCBI database. Of the 57 double-hit SNPs, we carried out SNP-typing in 94 Africans, 96 Asians, and 83 Caucasians and found striking ethnic differences in SNP frequencies and haplotype evolution. Four BAC-transgenic mouse lines, carrying the human CYP1A2 and much of the spacer region (except 8,115 bases closest to the CYP1A1 gene), expressed only negligible basal CYP1A2 mRNA, which was not inducible by dioxin. A fifth BAC-transgenic mouse line, carrying both the human CYP1A1 and CYP1A2 genes and ample amounts of 3 flanking sequences, plus all of the spacer region in the absence of the mouse *Cyp1a1* or *Cyp1a2* genes expressed the human CYP1A1 and CYP1A2 mRNA, protein and enzyme activities in liver and various nonhepatic tissues very similar to that of the mouse. Comparison of this hCYP1A1_1A2 transgenic line with hCYP1A1_1A2 lines carrying other common human haplotypes will enable us to evaluate function in human CYP1A1_CYP1A2 locus variability, with regard to polycyclic aromatic hydrocarbon- and aromatic amine-induced toxicity and cancer.

Establishment and characterization of *Aire*-expressing thymic cell lines. *Y. Yamaguchi*¹, *J. Kudoh*¹, *K. Tani*¹, *K. Sakai*¹, *S. Noda*², *A. Takayanagi*¹, *N. Shimizu*¹. 1) Department of Molecular Biology, Keio University School of Medicine, Tokyo, Japan; 2) Department of Nursing, School of Health Science, Tokai University, Kanagawa, Japan.

Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED: OMIM 240300) caused by mutation in autoimmune regulator (AIRE) gene is an organ-specific autoimmune disease with monogenic autosomal recessive inheritance. Recently, aberrant or promiscuous expression of a broad range of peripheral tissue-restricted genes by thymic medullary epithelial cells has been implicated in the essential process of establishing self-tolerance and AIRE in thymic epithelial cells has been demonstrated, by experiments using knockout mice, to control this promiscuous gene expression. Since AIRE is expressed only in a few epithelial cells of thymus and not expressed in established thymic epithelial cell lines, biochemical analysis of AIRE function in thymic epithelial cells is very difficult, and thus little is known about AIRE function associated with the mechanism regulating the transcription of peripheral tissue-restricted genes in thymic medullary epithelial cells, although AIRE protein has been reported to have both transcriptional activator and E3 ubiquitin ligase activities. To establish permanent lines derived from thymic medullary epithelial cells which express AIRE gene, we produced transgenic mice expressing SV40 T-antigens driven by mouse *Aire* gene promoter. Gross abnormality was found only in thymus of transgenic mice, namely they developed gigantic thymus about 40 days after birth. RT-PCR analysis revealed that SV40 T-antigen mRNA is expressed in the gigantic thymus but not in any other tissues examined. Cells dissociated from the gigantic thymus were cultured and three cell lines were cloned by limited dilution. These established cell lines (*Aire* cell) expressed *Aire*, SV40 T-antigen, and some peripheral tissue-restricted genes against which antibodies were produced in the APECED patient. *Aire* cell lines will be invaluable for the biochemical analysis of *Aire* gene function in relation to the promiscuous gene expression in thymic epithelial cells.

Regulation of Hermansky Pudlak Syndromes genes in human lymphoblastoid cell lines. A. Rivera, C. Carmona-Rivera, C.L. Cadilla. Department of Biochemistry, University of Puerto Rico School of Medicine, San Juan, PR.

The Hermansky Pudlak Syndrome (HPS)[MIM#203300] is the major cause of albinism cases in Puerto Rico. HPS is caused by defects in many genes, seven of them have been identified in humans. *HPS2* codes for the 3A subunit of the AP3 adaptor complex and *HPS7* codes for dysbindin, a component of the dystrophin associated protein complex (DPC), the rest of the human genes are novel and show no sequence similarity to other proteins and have few known functional domains. The HPS gene products are frequently undetectable in cells or tissues derived from both human patients and mouse models of HPS. The expression of the *HPS1*, *HPS2*, *HPS3*, *HPS4*, *pallidin* and *muted* genes in lymphoblast cell lines was examined by RT-PCR. The mRNA^{HPS1} was detectable in cells derived from a patient with HPS-1. Expression of the *HPS1*, *HPS2*, *HPS3*, *HPS4*, *pallidin* and *muted* genes was detectable by RT-PCR in both normal and HPS-1 human lymphoblast cell lines. Analysis of the 5-flanking DNA of several HPS genes using the TESS software showed potential response elements for glucocorticoid hormones, cAMP and Phorbol esters. Preliminary RT-PCR analysis of serum-starved cells treated with dexamethasone (DEX), PMA, cAMP, and Insulin for 8 hours showed an enhancement in the expression of almost all HPS genes. Real time RT-PCR analysis confirmed the inductive effect of these agents on the expression of HPS genes, with the exception of the *HPS3* gene, whose mRNA levels were decreased by cAMP treatment. Northern blot analysis shows an inductive effect of Dexamethasone, cAMP and Insulin on the expression of *HPS2*. However, treatment with PMA caused a decrease in the mRNA^{HPS2} levels. Western Blot analysis of lymphoblasts treated with PMA indicated a slight reduction in the expression of HPS1 and HPS4 proteins in normal human lymphoblasts. Further studies on the regulation of HPS genes are necessary to fully understand the cellular pathways affected in HPS. Grants from NIGMS S06GM08224 and R25GM61838 and NCRRG12RR03051 supported this study.

FMRP binds mainly to mRNAs relevant to neurodevelopment: Use of a yeast tri-hybrid approach to analyze protein-mRNA binding. *N. Zhong*^{1,2}, *K. Zo*², *A. Wronska*¹, *W. Ju*¹, *J. Shen*¹, *W.T. Brown*¹, *Y. Shen*². 1) Department of Human Genetics, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY; 2) National Key Laboratory of Molecular Medical Biology, China Academy of Medical Sciences/Peking Union Medical College, Beijing, China.

Lack of FMRP, the FMR-1 encoded protein, clinically results in the fragile X syndrome. FMRP has been characterized as having intrinsic mRNA-binding activity. To isolate and characterize FMRP-bound mRNA molecules, we employed a yeast tri-hybrid system. An RNA-expressing cDNA library was constructed in the pRH5 plasmid using human brain tissue. A full-length human FMR1 cDNA, that expresses FMRP in-frame, was subcloned into pYES3. Library screening was carried out by filter assay on selective medium with 3-AT. Of more than 50 mRNA molecules isolated, the majority was relevant to neuron development, including NEDD5, ROBO1, GDF11, and calmodulin 2. In addition, there were four categories of RNAs involved either in regulating cellular transcription and translation, or ribosomal transcripts, or RNA-binding protein transcription, as well as transcripts with uncharacterized functions. Characterization of these RNAs expression profiles in *fmr-1* knockout mouse brain by quantitative PCR and in situ hybridization are currently being undertaken. This approach has the potential to open new avenues to explore the pathogenic mechanism underlying the fragile X syndrome. It may also contribute new therapeutic targets for treatment of the syndrome.

Comparative genomics identifies a flagellar and basal body proteome that includes the *BBS5* human disease gene. *J.M. Gerdes*¹, *J.B. Li*³, *C.J. Haycraft*⁴, *Y. Fan*⁵, *T.M. Teslovich*¹, *H. May-Simera*⁶, *H. Li*⁷, *O.E. Blacque*⁵, *C.C. Leitch*¹, *R.A. Lewis*⁸, *J.S. Green*⁹, *P.S. Parfrey*⁹, *M.R. Leroux*⁵, *W.S. Davidson*⁵, *P.L. Beales*⁶, *L.M. Guay-Woodford*⁷, *B.K. Yoder*⁴, *G.D. Stormo*³, *S.K. Dutcher*³, *N. Katsanis*^{1,2}. 1) McKusick-Nathans Institute of Genetic Medicine; 2) Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD; 3) Department of Genetics, Washington University School of Medicine, St. Louis, MS 63110; 4) Department of Cell Biology, University of Alabama at Birmingham, Birmingham, AL 35294; 5) Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC V5A 1S6, Canada; 6) Molecular Medicine Unit, Institute of Child Health, University College London, London WC1N 1EH, UK; 7) Division of Genetic and Translational Medicine, Departments of Medicine, Pediatrics, and Genetics, University of Alabama at Birmingham, Birmingham, AL 35294; 8) Departments of Molecular and Human Genetics, Ophthalmology, Pediatrics, and Medicine, Baylor College of Medicine, Houston, TX 77030; 9) Departments of Medical Genetics and Clinical Epidemiology, Memorial University, St. John's Newfoundland, Canada.

Cilia and flagella are microtubule-based structures nucleated by modified centrioles termed basal bodies. These biochemically complex organelles have more than 250 and 150 polypeptides, respectively. To identify the proteins involved in ciliary and basal body biogenesis and function, we undertook a comparative genomics approach that subtracted the nonflagellated proteome of *Arabidopsis* from the shared proteome of the ciliated/flagellated organisms *Chlamydomonas* and human. We identified 688 genes that are present exclusively in organisms with flagella and basal bodies and validated these data through a series of *in silico*, *in vitro*, and *in vivo* studies. We then applied this resource to the study of human ciliation disorders and have identified *BBS5*, a novel gene for Bardet-Biedl syndrome. We show that this novel protein localizes to basal bodies in mouse and *C. elegans*, is under the regulatory control of *daf-19*, and is necessary for the generation of both cilia and flagella.

Investigating NAD kinase as a mitochondrial mutator. *K.V. Shianna*¹, *M.K. Strand*², *D.A. Marchuk*¹. 1) Dept Mol Genetics/Microbiol, Duke Univ Medical Ctr, Durham, NC; 2) Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

The aging process, along with many neurodegenerative disorders, results in an increase in the mitochondrial mutation frequency. The molecular mechanisms behind this are not fully understood. We have previously shown that insertional inactivation of the yeast mitochondrial NADH kinase (*POS5*) leads to a 50-fold increase in the mitochondrial mutation rate. Expression analysis on the *pos5* demonstrated increased expression of most of the iron transport genes. This expression pattern is very similar to the yeast frataxin homolog knockout, a model for the neurodegenerative disease Friedreich's ataxia. In addition, the *pos5* showed decreased expression for many of the enzymes involved in electron transport. We have demonstrated that the *utr1* (cytoplasmic NAD kinase) *pos5* double knockout is synthetically lethal in yeast, and that this lethality can be rescued by the human NAD kinase cDNA. Since the NAD/H kinase proteins are highly conserved, we hypothesized that mutations in a mammalian homolog could lead to an increased mitochondrial mutation rate. Recently, we have established an NAD kinase mouse model. The homozygote is embryonic lethal. The F1 and F2 heterozygotes appear to be healthy at six months of age. We are currently measuring the frequencies of mitochondrial deletions and point mutations. Crosses are also being performed to determine if the heterozygote state has an effect on the mouse after several generations of maternal mitochondrial inheritance. Also, the heterozygote is being coupled with other knockouts that affect responses to reactive oxygen species.

Complementation Studies of Shwachman-Diamond Syndrome Protein Homologues in Yeast. *G.R.B. Boocock*^{1,2}, *M.R. Marit*¹, *C. Shamma*³, *A.J. Warren*³, *J.M. Rommens*^{1,2}. 1) Program in Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, ON, Canada; 2) Department of Molecular and Medical Genetics, University of Toronto, Toronto, ON, Canada; 3) MRC Laboratory of Molecular Biology, Cambridge, UK.

Shwachman-Diamond syndrome (SDS) is a recessive, multisystem disorder affecting the bone marrow, exocrine pancreas, and skeleton. The gene that is mutated in SDS (*SBDS*) encodes a soluble protein with highly conserved counterparts in all eukaryotic and archaeal genomes. The structure of the *Archaeoglobus fulgidus* homologue has recently been solved and revealed a three-domain arrangement, with a novel amino-terminal domain, a central domain composed of three α -helices, and a carboxyl-terminal domain that occurs in a number of proteins of diverse function. *YLR022c*, the *Saccharomyces cerevisiae* homologue of *SBDS*, is an essential gene. We have developed a complementation assay in yeast based on a plasmid shuffle system. When 17 disease-relevant missense mutations were generated in *YLR022c*, all but two over-expressed variants supported yeast growth, indicating that most mutations do not abrogate protein function. This is in agreement with the absence of homozygosity for common, early truncation mutations in a large patient collection, indicating that SDS arises due to hypomorphic alleles of *SBDS*, and that complete loss of *SBDS* function is incompatible with life. We have determined that *SBDS* itself cannot functionally substitute for *YLR022c*. However, chimeras containing *SBDS* sequence corresponding to domain-1 or domain-3 were capable of complementation. A chimera with the carboxyl-terminus of *Arabidopsis thaliana*, including domain-3 and an extended segment with a putative zinc finger, was also capable of complementation. Functional complementation did not extend to the Archaea, as neither domain-1 nor domain-3 from *Solfolobus solfataricus* could substitute for homologous yeast sequences. Taken together, these results suggest that domain-2 imparts a species-specific functionality, while domains 1 and 3 are interchangeable amongst eukaryotes. Study of *YLR022c* will therefore yield direct insights into the function of *SBDS*.

The Diamond Blackfan Anemia gene product, ribosomal protein S19, interacts with the Pim-1 oncoprotein. I.

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Diamond Blackfan Anaemia (DBA) (MIM 205900) is a congenital disease characterized by defective erythroid progenitor maturation. The bone marrow progenitors from patients do not respond in vitro and in vivo to exposure to erythropoietic growth factors, such as EPO, IL-3, TPO. Mutations in the gene encoding for ribosomal protein (rp) S19 account for 25% of patients. The link between defective erythropoiesis and rps19 is still unclear. Two hypotheses, not mutually exclusive, have been proposed: one considers that the cause of the defective erythropoiesis resides in a decreased protein synthesis, whereas the second invokes the loss of a possible extraribosomal function. In recent years several ribosomal proteins have been shown to have a second function besides their structural role in the ribosome. To identify a possible second role for rps19 we performed the Yeast Two Hybrid System, using rps19 as the bait to screen a cDNA library obtained from human liver at the 19-24th week of gestation, a period in which hepatic erythropoiesis is active. In our hypothesis the nature of the interactor could have suggested a second role for rps19. Our screening identified a cDNA clone, present in three different versions, which encoded for the Pim-1 oncoprotein. PIM-1 is widely expressed in erythropoiesis after induction by erythropoietic growth factors, such as EPO, IL-3 and TPO. The interaction between Pim-1 and rps19 was demonstrated both in vitro and vivo, by a pulldown and a coimmunoprecipitation assay. Our data also show that both the N-terminal and C-terminal regions of rps19 are critical for this interaction and thus suggest a conformation site. The nature of this interaction and its functional role in hemopoiesis still need to be elucidated. In conclusion, a link between erythropoietic growth factor signaling and rps19 has been for the first time identified: these data suggest a new biological role for rps19.

Spastin shows association with the centrosome: implications for the pathogenesis of Hereditary Spastic

Paraplegia. *M.T. Kloos¹, I.K. Svenson¹, A.C. Horton², A.A. Ashley-Koch³, M.A. Pericak-Vance³, M.D. Ehlers², D.A. Marchuk¹.* 1) Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC; 2) Department of Neurobiology, Duke University Medical Center, Durham, NC; 3) Center for Human Genetics, Duke University Medical Center, Durham, NC.

Hereditary Spastic Paraplegias (HSPs) represent a group of genetically heterogeneous neurodegenerative disorders characterized primarily by hyperreflexia and progressive spasticity of the lower limbs. The common pathology is neuronal degeneration that is maximal at the distal ends of the longest axons of the central nervous system. Mutations in the *SPG4* gene, encoding spastin, result in the most common form of autosomal dominant HSPs. To gain insight into the normal function of spastin and its role in HSP pathogenesis we have developed a peptide antibody to investigate the subcellular localization of endogenous spastin. The peptide antibody was designed for maximum specificity to all spastin isoforms. The epitope is located outside of the highly conserved AAA domain, outside the less conserved MIT domain, and not encoded by alternatively spliced exons 4, 8 or 15. Our data suggest that endogenous spastin in several cell types is localized to the centrosome, the site of microtubule nucleation and organization. A location at the microtubule organizing center (centrosome) is consistent with published reports showing that mutant spastin associates with and reorganizes microtubules in transient transfection experiments. Taken together, our findings are consistent with a proposed cytoplasmic role for spastin in regulation of microtubule dynamics. These findings are also consistent with the proposed axonal transport theme for HSP pathogenesis, as the microtubules form the molecular rail system for axoplasmic flow.

Biological and Clinical Consequences Of Sarcoglycan Gene Mutations. *M. Hegde¹, Y.M. Chan², B. Chong¹, S.V. Khadilkar⁴, W. Shi², M. Farber², V. Wong³, P. Ip³, Z. Simmons⁵, C.S. Richards⁶, D.R. Love⁷.* 1) Medical Genetics Laboratories, Baylor Col Medicine, Houston, TX; 2) The Henry Hood Research Program, Sigfried and Janet Weis Center for Research, The Geisinger Clinic, Danville, PA 17822; 3) Division of Child Neurology, Department of Pediatrics and Adolescent Medicine, Queen Mary Hospital, University of Hong Kong, Hong Kong SAR, China; 4) Department of Neurology, Bombay Hospital, Bombay, India; 5) Department of Neurology, Penn State College of Medicine, Milton S. Hershey Medical Center, Hershey, PA 17033; 6) OHSU DNA Diagnostic Lab OHSU3181 SW Sam Jackson Park Road, MP-350 Portland, OR 97239; 7) School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand.

Mutations in sarcoglycans have been shown to cause the autosomal recessive Limb-girdle Muscular Dystrophy (AR-LGMD). Sarcoglycans consist of four transmembrane proteins (a-SG, b-SG, g-SG and d-SG) and form a specific complex in sarcolemma. We have evaluated 100 clinically defined LGMD patients from US, New Zealand, Hong Kong and India. To screen for all the sarcoglycan genes involved in LGMD, a mutation scanning strategy using dHPLC and/or sequencing of the coding region, with immunohistochemical analysis of muscle biopsies was used. We identified mutations in 40 patients, of which 21 are missense mutations, 10 are nonsense mutations and 9 are frame-shift mutations. Earlier we have demonstrated that assembly of the sarcoglycan complex follows a pathway that is dependent on the interaction between b-SG and d-SG. In co-expression studies using truncated deletions in d-SG, the extreme C-terminal was shown to be critical for interaction with b-SG. This domain consists of conserved EGF-like repeat that forms intramolecular di-sulfide bond as well as a highly conserved sequence of 8 amino acids. We predict that these sarcoglycan mutations produce deleterious effects on muscle function by disrupting the formation of a functional b/d-SG core during assembly process. The analysis of truncated deletions in sarcoglycans will help further elucidate the functional role of different domains of sarcoglycans and better understand the phenotypic variation in LGMD.

Regulation of Wilms Tumor Gene 1(WT1) in desmoid tumors. *S. Amini Nik¹, P. Hohenstein², A. Jadidizadeh¹, K. Van Dam¹, A. Bastidas¹, R. Berry³, C. Patek³, B. Van der Schueren¹, J. Cassiman¹, S. Tejpar¹.* 1) Department of Human Genetics, University of Leuven, Leuven, Belgium; 2) Comparative and Developmental Genetics, MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh, EH4 2XU, UK; 3) Cancer Research UK Laboratories, Molecular Medicine Centre, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK.

Desmoid tumors (aggressive fibromatosis) are locally invasive soft tissue tumors in which b-Catenin/TCF3 mediated Wnt signaling is activated. More than 80% of desmoid tumors contain activating mutations in b-Catenin. It has been shown that the Wnt signaling pathway interacts with WT1 in normal kidney development and plays a role in the genesis of some Wilms tumors. About 15% of Wilms tumors contain WT1 mutations and of these, about 50% contain b-Catenin mutations. This overlap in mutation pattern of WT1 and b-Catenin in Wilms tumor suggests that these two genes may collaborate in the genesis of a subset of Wilms tumors. To investigate whether this hypothesis could be extended to other Wnt-dependent tumor types, we searched for WT1 mutations and studied WT1 expression in b-Catenin mutant desmoid tumors. We investigated the expression of WT1 mRNA and protein in desmoid tumors. Medium to high abundant levels of WT1 mRNA were detected by TaqMan quantitative PCR in all tested desmoids cells, while adjacent normal fibroblasts showed less expression of WT1. Western blot analysis and immunohistochemistry confirmed this overexpression at the protein level. A mutational screen of the WT1 zinc-finger region by sequence analysis did not identify any mutations. Finally, we investigated a possible role of b-Catenin on WT1 regulation and vice versa. Overexpression of different b-Catenin mutants in the HEK293T cell line did not modulate WT1 promoter activity and WT1 did not affect b-Catenin /TCF transcriptional activity in this cell line. These results show that the wild-type WT1 gene is strongly overexpressed in b-Catenin mutant desmoid tumors and may play a role in tumorigenesis of desmoid tumors, similar to what has been suggested in some epithelial malignancies.

A yeast based mutation screen to uncover new insights into frataxin function. *G.L. Dalgliesh, S.M. Purandare, K.G. Marichal, J.M. Brown, S.I. Bidichandani.* Depts of Biochemistry & Mol. Biol. & Pediatrics. Univ of Oklahoma Hlth Sci Ctr, 975 NE 10th St, Oklahoma City, OK 73104, USA.

The inherited neurodegenerative disease Friedreich ataxia (FRDA) is caused by loss of frataxin function due to either a GAA triplet repeat expansion in intron 1 of the *FRDA* gene or, very rarely, a point mutation in the coding sequence. Little is known about the functionally important residues of this highly conserved protein, despite the gene sequence and protein structure of frataxin being known. We exploited this high degree of conservation by constructing a chimeric yeast-human frataxin (*cYHF*) allowing mutations in the human *FRDA* sequence to be analyzed in a yeast model system. Mutation libraries were generated by amplifying *cYHF* using two error prone polymerases providing a comprehensive spectrum of mutations. PCR conditions were adjusted so the majority of mutant clones had a single base substitution. To determine the effect of these mutations on frataxin function, a yeast based genetic screen was developed using a heterozygous yeast frataxin knockout. This heterozygote has a normal phenotype, but when sporulated, the haploid knockouts are respiratory deficient. The knockout was used to screen both mutation libraries for functionally important mutations based on the inability of the library plasmid to rescue the respiratory deficient phenotype. We have screened 4,000 clones to uncover thirteen novel missense mutations. These mutations were found to be in a similar region of the protein as patient mutations i.e. on either the beta sheets or second alpha helix of frataxin, encoded by exons 4&5. Notably, five of the mutations are in the final carboxy tail of frataxin, an area which has not previously been implicated in frataxin function through patient mutations. The effects of these novel mutations on frataxin expression and yeast sensitivity to oxidative stress have also been measured. These measurements indicate that all mutations result in greater sensitivity to oxidative stress. The discovery and functional analysis of these mutations has yielded insight into functionally important regions of human frataxin, including a region not previously recognized.

Improved quantitative PCR-based gene dose analysis for the clinical laboratory: Assay design parameters and quality assurance metrics. *B.C. Hendrickson, T. Judkins, A. Kouzmine, J. Tazelaar, K. Eliason, D. Gonzales, B.E. Ward, T. Scholl.* Myriad Genetics, Salt Lake City, UT.

Gene dosage analysis is important in clinical genetic testing for detection of duplication and deletion events that impair the structural integrity of genes. For many clinical laboratories, quantitative PCR approaches are preferred due to simplicity of specimen isolation and assay implementation. However, quantitative measurements by PCR are a demanding application of this technology.

A number of quantitative PCR approaches, including real-time and single point analysis of multiplexed reactions, have been proposed for rearrangement detection. Presented are comparative data on several available technologies. Also, the benefits to assay performance by employing various design parameters relating to amplicon and primer design, and redundancy of analysis are demonstrated in a model system that involved a comprehensive assay for gene-dosage analysis of *BRCA1* and *BRCA2*. When compared to quantitative methods currently in use, assay robustness was increased by applying the following improvements. All regions were represented by at least two unique amplicons, each in separate multiplexed reactions. Adjacent exons were integrated into different multiplexes. Within multiplexes, amplicons for both genes were interleaved along with control genes selected from haplo- and duplolethal regions of the genome. Also, a likelihood based approach rather than naïve dosage quotients or visual inspection was demonstrated to be superior for data analysis. Using historical data, patient results for each multiplex are normalized to every possible mutation scenario and the mutation status is returned with a statistical level of confidence. Finally, application of this model system to 500 anonymous high risk breast/ovarian cancer patients negative by sequence analysis demonstrated that 6% had large rearrangements. The application of these methods permits quantitative PCR assays with increased robustness and quantitative measures of performance that improve application of this technology to clinical genetic testing.

Both in vivo and in vitro evidence of inactivation of splice donor site by G to A nucleotide change at 5th nucleotide of intron 32 of the dystrophin gene. *T.Thi. Hoai Thu*¹, *A. Surono*¹, *M. Yagi*¹, *Y. Takeshima*¹, *H. Wada*², *M. Matsuo*¹. 1) Department of Pediatrics, Kobe University Graduate School of Medicine, Japan; 2) Department of Pediatrics, Sakura Ryoikuen Hospital, Sanda, Japan.

Maturation of pre-mRNA having a single nucleotide change within consensus sequences of splice donor site is controversial. A single nucleotide change at 5th nucleotide of intron 32 of the dystrophin gene (GIVS32+5A) has been reported either a polymorphism or a mutation identified in DMD. Here we show both in vivo and in vitro evidence that GIVS32+5A inactivate an authentic splice donor site and instead activate cryptic splice donor sites. In a Japanese DMD case dystrophin mRNA in lymphocytes was found to have two novel mRNAs; one had 98nt deletion of exon 32 and the other had 28 nt insertion between exons 32 and 33. However, only one nucleotide change of G to A conversion at 5th nucleotide of intron 32 of the dystrophin gene (GIVS32+5A) was disclosed from genomic DNA analysis. In order to exemplify inactivation of splice donor site by GIVS32+5A splicing products from mini-dystrophin genes with either normal or mutant intron 32 sequence in Hela cells were analyzed by reverse-transcription PCR amplification. Remarkably 98bp deletion of exon 32 was identified in mutant mini-gene transcript, while full of exon 32 sequence was present in the mini-gene transcript with normal intron 32 sequences. Our results indicated clearly that GIVS32+5A inactivated the authentic splice donor site and activated the cryptic splice donor site. In addition, it was shown that 23 mer RNA probe corresponding to the consensus and its neighboring sequences of splice donor site was shown to bind nuclear protein stronger than the RNA probe with the mutant sequence. It was concluded that GIVS32+5A was a cause of inactivation of splice donor site and produce two out-of-frame dystrophin mRNAs responsible DMD phenotype though GIVS32+5A was once reported as a polymorphism. This report shows that in vitro splicing reaction can be used for identification of a disease causing mutation.

Identification of PXR/RXR transcriptional targets by *in vitro* competition with DNA binding sites predicted by information theory. C. West¹, C. Bi^{1,2}, P.K. Rogan^{1,2}. 1) Children's Mercy Hospital, Schools of Medicine and; 2) Computer Science and Engineering, University of Missouri-Kansas City.

PXR/RXR is a generalized chemosensor that induces expression of metabolic and transporter proteins by regulating their corresponding gene promoters. The affinity (ie. individual information content, R_i) of PXR/RXR for cognate enhancer elements (PXREs) can be predicted by information theory-based DNA binding site models. We previously refined a model of PXRE sequences by iteratively cycling information theory-based prediction and validation using published and predicted binding sites. Here, we use a novel microtiter plate assay to specifically detect binding sites with defined information contents from putative PXR/RXR transcriptional targets. The amount of PXR/RXR bound to a solid-phase, reference nucleic acid binding site (eg. CYP3A4 proximal PXRE [$R_i=16.5$ bits]) is determined for varying concentrations of a test competitor sequence in solution. Protein bound to the reference sequence is detected with an enzyme-linked, secondary antibody that recognizes a primary antibody to RXR. To identify sites with a broad range of binding affinities, the assay is performed with a series of reference and competitor oligonucleotides with comparable R_i values. Published (n=4) and predicted (n=4) binding sites used to develop the refined model were confirmed with this assay. Lower concentrations of stronger competitor sequences can sequester PXR/RXR, decreasing the amount of protein bound to the reference sequence. Controls included no competitor, multiple concentrations of a homopolymer negative control sequence, and the consensus binding site predicted by information theory. The assay was validated with a set of 15 PXREs from rifampin-inducible genes that were not included in the original model. Finally, we evaluated 12 strong binding sites detected in information theory-based scans of known human gene promoters, and demonstrated that each of these sites compete with the reference site for binding to PXR/RXR. Most of the loci associated with these sites are novel targets and are not related to genes known to be involved in xenobiotic metabolism or transport.

Quantitative studies of *AGTR1* mRNA haplotypes and metabolic syndrome traits. *M.R. Abdollahi, R. Lewis, T.R. Gaunt, S. Rodriguez, H.E. Syddall, C. Cooper, D.I.W. Phillips, I.T. Cameron, I.N.M. Day.* Human Genetics Division (MP808), Southampton General Hospital, Southampton, Tremona Rd, SO16 6YD, United Kingdom.

Introduction: angiotensin II type I receptor (*AGTR1*) is the main receptor through which angiotensin II, the most important component of the renin angiotensin pathway, influences cardiovascular tone, cell growth, fluid and electrolyte balance. *AGTR1* is expressed in blood vessel walls, heart and many other organs. *AGTR1* A1166C, in the 3' UTR, has been reported to associate with hypertension and myocardial infarction. **Purpose:** to study the association of the *AGTR1* polymorphisms with cardiovascular and metabolic traits and investigate transcript expression. **Methods:** we used Phase and STATA to examine haplotypes of *AGTR1* constituted of A1166C, L191L, A-153G and C-521T in relation to anthropometric and metabolic syndrome phenotypes in the North and East Hertfordshire UK populations. Ratiometric analyses (based on quantitative-gel image analysis of restriction digests of cDNA) were developed for the transcribed SNPs A1166C and L191L in order to determine relative allelic expression from haplotypes. Additional to these within-individual-between-allele assays, between-individual comparisons were made using TaqMan assays applied to both homozygous and heterozygous genotypes and haplotypes, analysed by Phase, SPSS and HTR. **Results:** 1) CC genotype of *AGTR1* A1166C (and L191L) are significantly associated with protective anthropometric and metabolic syndrome trait values. 2) C-521T and A-153G did not reflect these associations. 3) There are two haplotype blocks in the *AGTR1* gene. 4) In the quantitative mRNA assays, there was no significant difference for alleles of L191L, but 1166C and haplotypes carrying it marked low *AGTR1* mRNA level. Effect was much greater in CC homozygotes than in heterozygotes. **Conclusion:** *AGTR1* 1166C marks anthropometric and metabolic effects and also marks low *AGTR1* mRNA levels. All effects were much more prominent in homozygotes, possibly reflecting interallelic interaction through feedback loops of mRNA regulation.

Functional polymorphisms in the regulatory region of genes implicated in G1/S checkpoint. *H. Belanger, P. Beaulieu, M. Lariviere, C. Moreau, S. Langlois, D. Labuda, D. Sinnett.* Division of Hematology-Oncology, Sainte-Justine Hospital Research Center, University of Montreal, Montreal, QC, Canada.

Cell cycle checkpoints mediate quality control of the cell cycle, particularly the fidelity of replication. Following abnormalities, these checkpoint mechanisms will stop the cell cycle progression and activate repair processes or cell death by apoptosis. We propose that functional polymorphisms in candidate genes could bring variability in the mechanisms that regulate the cell cycle which could give rise to differences in the risk of developing complex diseases such as cancer. The main objectives are to identify and validate functional polymorphisms in targeted regulatory regions, arbitrary defined as a 2kb genomic segment upstream of exon 1. Mutation analysis was carried out on 17 genes involved in the G1/S checkpoint. Polymorphisms were detected using denaturing HPLC in a population panel of 40 individuals from different ethnic backgrounds. A total of 136 regulatory SNPs (rSNPs) were identified. In this study we selected 13 rSNPs that were predicted using *in silico* tool, to lead to gains of binding sites for transcription factors. Electrophoretic mobility shift assays showed that nuclear proteins from different cell lines (HeLa, Caco2, Jeg-3, HepG2) exhibited differences in the binding affinity for 12 of the rSNPs tested. Expression analyses were performed by cloning 2kb haplotypes corresponding to different construction of the selected rSNPs into a luciferase reporter gene vector (pGL3) and were tested for differences in their ability to drive transcription in HeLa, Jeg-3 and HepG2 cells. Differences in expression greater than 1.5-fold were detected between haplotypes of TGF1, E2F1, CDC25a and HDAC1. However, no significant differences were found between haplotypes for p21, p27 and RB1. The detection and validation of polymorphisms in the regulatory regions of genes implicated in cell cycle checkpoints will provide a better understanding of their involvement in susceptibility to complex diseases. This work is supported by genome-Quebec/Canada.

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In-vitro studies of natural mutations in PK-M2 isozyme. *M.K. Akhtar, A. Dhir, R. Bamezai.* NCAHG, SLS, Jawaharlal Nehru University, New Delhi, Delhi, India.

No natural mutations in Pyruvate kinase (PK)-M2 gene have been implicated in any disease. For the first time natural mutations were reported from our laboratory in PK-M2 gene in Bloom Syndrome cell lines and the Lymphocytes of a Bloom Syndrome patient from India. Two different missense mutations, H391Y and K421R were found in the region of Inter Subunit Contact Domain, which down regulates the enzyme activity to different extents. However, the biological significance of these mutations is not yet clear. Transcriptional and translational regulation of PK-M2 gene were assessed by Real time PCR and enzyme activity in PHA-stimulated Lymphocytes from normal volunteers at different time points. The RNA level increased many fold at 12hr and decreased at 24hr when compared to 0hr, and the specific activity decreased at 12hr, which increased again at 24hr to a level similar to 0hr. Normal PK-M2 cDNA and its mutants were expressed in bacteria and found that the specific activity of H391Y mutant decreased by about 50% and in K421R mutant, decreased by about 70% as compared to normal PK-M2. Further these were sub-cloned in EGFP expression vector, transfected into HepG2 cell line. Confocal analysis revealed that EGFP vector alone could go both in cytosol and in nucleus, while EGFP-PK-M2 remained in cytosol around the cell periphery. The H391Y and K421R mutants localised both in cytosol and in the nucleus. Transcriptional and translational studies showed that there is certain time lag between the appearance of PK-M2 transcript and functionally active PK-M2 molecules. The two different point mutations changed the normal structure of PK-M2, which not only decreased the activity of the enzyme to different levels but also affected the property of localization within a normal cell as observed in Confocal studies. It will be interesting to know if the mutant PK-M2 proteins enter into the nucleus due to a loss of identity or any structural change made the protein to interact with any other partner protein which facilitated its entry into the nucleus.

Identification of potential downstream genes of Trps1 by cDNA microarray analysis of mouse embryonic limbs.

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Mutations in the TRPS1 gene cause the tricho-rhino-phalangeal syndrome, which is characterized by craniofacial and skeletal abnormalities. The gene encodes a transcription factor that has a GATA-binding zinc finger and represses GATA-regulated gene expression. During development, the gene is highly expressed in the cartilage condensations of the limbs and in other structures (Kunath *et al.* Gene Expression Patterns 2002). The phenotype of Trps1 knockout mice that are heterozygous for an in frame deletion of the GATA-zinc finger coding exon is comparable to but milder than the human TRPS I. Homozygous knockout mice die shortly after birth (Malik *et al.* MCB 2002). To identify genes affected by the Trps1 knockout, we used the MOE430A Affymetrix microarrays to determine the expression profiles of E11.5 limbs from normal and mutant mice. We compared the expression profiles of 3 wild type, 2 heterozygous and 3 homozygous knockout mice from the same litter. Genes were considered as differentially expressed when showing at least a 1.5-fold difference in the mean expression level between wild type and mutant embryos with a standard deviation of less than 25%. Between heterozygous and wild type embryos we found 329 differentially expressed genes: 211 overexpressed in the mutants and 118 in the wild type. When comparing homozygous and wild type mice, we found 177 differentially expressed genes: 75 overexpressed in the mutants and 102 in the wild type. Thirty-four genes were conspicuous in both comparisons. Among the differentially expressed genes were genes for transcription factors, growth factors and extracellular matrix components that are known to play important roles in mouse embryonic limb development. Our results provide a first insight into the complex role that Trps1 plays in mouse limb development.

Cross-species sequence comparison of alpha-fetoprotein gene regulation. *J.N. Davidson, M. Glenn, L. Long, A.A. Wells, B.T. Spears.* Microbiology, Immunology and Molecular Genetics, University of Kentucky, Lexington, KY.

The alpha-fetoprotein (AFP) gene is expressed at high levels in the fetal liver but is dramatically repressed at birth. The gene is normally silent in the adult liver but can be reactivated in hepatocellular carcinomas and during liver regeneration following liver damage. Three upstream enhancers, E1, E2 and E3, regulate transcription of the AFP gene both in mouse and rat. Sequence analysis indicates that E1 and E2 are more closely related to each other than either is to E3, suggesting that E1 and E2 arose from duplication of a primordial enhancer. The purpose of this study was to compare DNA sequence data for the upstream region of the AFP genes from a variety of mammals. Data originated from genomic databases as well as from our own sequencing results for horse. Sequences were then aligned and analyzed. In primates, dog and horse, we found a deletion within E1 that eliminates nearly the entire enhancer, and the human E1 is inactive. E2 is the most conserved of the three enhancers. The E3 sequence is dramatically different across all species studied. Moreover, human and mouse E3 bind different transacting factors. These results indicate species-specific differences in AFP regulation. Data will also be presented that describes a novel hexanucleotide repeat (CTATAT) that is present in humans, chimpanzees and gorillas but absent in mice and rats. Among humans repeat length and surrounding sequences are highly polymorphic. Supported by NIH grant DK51600.

The mouse dystrophin muscle enhancer-1 imparts skeletal muscle, but not cardiac muscle, expression onto the dystrophin Purkinje promoter in transgenic mice. *Y. De Repentigny¹, P. Marshall¹, R.G. Worton^{1,2,4}, R.*

Kothary^{1,3,4}. 1) Molecular Medicine Program, Ottawa Health Research Inst, Ottawa, Ontario, Canada; 2) Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Ontario, Canada; 3) Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Ontario, Canada; 4) Department of Medicine, University of Ottawa, Ottawa, Ontario, Canada.

A subset of patients harboring mutations in the dystrophin gene suffer from X-linked dilated cardiomyopathy (XLCM), a familial heart disease that is not accompanied by any clinical signs of skeletal muscle myopathy. Since the muscle (M) isoform of dystrophin is not expressed in these patients, the absence of skeletal muscle myopathy has been attributed to expression of the brain (B) and cerebellar Purkinje (CP) isoforms of dystrophin in skeletal, but not cardiac, muscles of XLCM patients. The compensatory mechanism of B and CP promoter upregulation is not known but it has been suggested that the dystrophin muscle enhancer from intron 1, DME-1, may be important in this activity. Previous studies have shown that the presence of the DME-1 is essential for a significant increase in B and CP promoter activity in skeletal muscle cells in culture. Here, we demonstrate that the mouse dystrophin CP promoter drives expression of a *lacZ* reporter gene specifically to the cerebellar Purkinje cell layer but not to skeletal or cardiac muscle of transgenic mice. However, if the mouse counterpart of DME-1 is present in the transgene construct, the CP promoter is now activated in skeletal muscle, but not in cardiac muscle. Our findings provide *in vivo* evidence for the importance of the dystrophin muscle enhancer sequences in activating the dystrophin CP promoter in skeletal muscle. Furthermore, they strengthen the model in which the DME-1 sequence activates the dystrophin B and CP promoters in the skeletal muscle of XLCM patients whereas the cardiac tissue remains refractory to this action.

Isoform repertoire and regulation of ectodysplasin (EDA) gene function. *T. Hashimoto, CY. Cui, M.C. Durmowicz, R. Nagaraja, D. Schlessinger.* Laboratory of Genetics, National Institutes of Health/National Institute on Aging, Baltimore, MD.

The EDA gene is mutated in anhidrotic ectodermal dysplasia in humans and in the Tabby mouse, resulting in the absence of sweat glands and several types of hair, as well as rudimentary teeth. The gene encodes a series of isoforms, but the complete catalogue of isoforms and their functional significance has remained unknown. Furthermore, a 2 kb DNA fragment just upstream of the transcription start site is sufficient to drive high level transcription of the gene in transfected cells in vitro, but the level of the mRNA is paradoxically very low in most cells in vivo, and might be related to differential formation or stability of various isoforms. To infer a more complete repertoire of EDA transcripts, we have derived cloned cDNAs from keratinocyte cells in which transcription is unusually high. The transcripts include a total of 9 species. In addition to the long EDA-A1 and -A2 forms, which jointly account for 77% of the transcripts, and 2 other previously reported transcripts (EDA-A3 and EDA-A4), five new transcripts were found including two that account for 7% and 2%, respectively. Isoform functions are largely unanalyzed, but we have shown that one of the longest species, EDA-A1, is able to correct some but not all of the EDA defects; and other species interact with EDA-A1, at least in the case of formation of one class of hair follicles. Finally, the possibility that mRNA stability might be linked to the level of expression of different isoforms was further hinted by the presence of AU-rich elements in the 3UTR that have been associated with instability of short-lived mRNA species. However, the half-life of cellular EDA is about 2 hr for all transcripts that have 4, 2, or no AU-rich elements; and this relative level of instability cannot explain the low expression of the gene in vivo compared to the strength of transfected promoter DNA. Instead, regulation appears to be at transcription for the entire range of isoform transcripts, through repressor action in most cells.

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Analysis of the 5 distal regulatory region of the human tyrosinase gene. *R.A King, J.P. Fryer, W.S. Oetting.* Dept. of Medicine and Institute of Human Genetics, Univ Minnesota, Minneapolis, MN.

BACKGROUND: Oculocutaneous albinism type 1 (OCA1) results from mutations of the tyrosinase gene. Most affected individuals are compound heterozygotes, and more than 150 different mutations of this gene have been described. Approximately 15% of OCA1 alleles have no detectable mutation with sequence analysis of the coding, adjacent intron, and proximal promoter regions of the gene suggesting that mutations in other regulatory regions of the gene may be responsible. A distal enhancer region of the tyrosinase gene has been identified (NCBI accession AY367052) (*Pigment Cell Res* 16:679, 2003). We have now extended our initial studies to identify additional components of this distal regulatory region and have identified a potential mutation in OCA1 that has functional effects on a reporter assay. **METHODS:** Constructs containing sequential additions or deletions of sequence from the original enhancer sequence (EPP2) were developed and analyzed in a luciferase expression system. Site directed mutagenesis was used to create specific base changes in the regulatory region sequence. **RESULTS:** Constructs with sequential deletion of the 5' end of the original EPP2 enhancer sequence produced a loss of enhancer activity, while the addition of contiguous sequence to the 5' end of EPP2 resulted in a significant increase in enhancer activity. Additional sequences further revealed a 5' negative regulatory element. Sequence analysis of the tyrosinase gene in individuals with OCA1 showed a single base substitution (c.-9322G>A), not found in 100 chromosomes from normally pigmented individuals with no history of OCA1. Expression analysis of the enhancer sequence containing this substitution resulted in a marked reduction in enhanced expression when compared to the wild type sequence. **CONCLUSION:** This work reveals the complexity of the tyrosinase distal regulatory region with enhancer and suppressor function, and identifies the first potential regulatory mutation of the tyrosinase gene that may be associated with the development of OCA1.

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The miscarriage-associated HLA-G -725G is associated with increased expression: Is too much of a good thing bad? *S.A. Kuldanek, C.M. Billstrand, K.A. Vigh, L.A. Weiss, C. Ober.* Human Genetics, The University of Chicago, Chicago, IL.

HLA-G is primarily expressed in trophoblast cells at the maternal-fetal interface, where it plays an immunomodulatory role in pregnancy. We previously identified in the Hutterites 18 SNPs, defining 8 unique haplotypes, in the 1300 bp upstream of exon 1, which contains all the known regulatory elements (Ober et al., 2003; *AJHG* 72:1425). One SNP, -725C/G, was associated with miscarriage (odds ratio in couples in which both partners carried a -725G allele = 2.76; $p=0.035$). To explore the hypothesis that -725G influences transcription rates, we subcloned the promoter region of 4 unique haplotypes (2 with -725G; 2 with -725C) into pGL3 vectors with a firefly luciferase gene, and transfected the vectors into a choriocarcinoma (trophoblast) cell line, JEG3. Assays of luciferase expression were run in triplicate in 8 independent experiments. Expression levels were significantly different between -725G and -725C haplotypes ($p=1.74 \times 10^{-6}$); median expression was 85% higher for haplotypes with -725G. Transferring by site-directed mutagenesis a G at -725 to a haplotype with -725C resulted in a significant change in expression ($p=1.22 \times 10^{-3}$), with median expression increasing 36% on the mutated haplotype. These studies indicate that the presence of a G at -725 results in significantly increased transcription, regardless of the haplotype background, and that overexpression of HLA-G in pregnancy may be a risk factor for miscarriage. Supported by HD21244.

THE TREACHER COLLINS SYNDROME GENE (TCOF1) PROMOTER CHARACTERIZATION. C.

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Treacher Collins syndrome (TCS) is an autosomal dominant craniofacial malformation, with an incidence of 1:50.000. The gene associated with this syndrome, *TCOF1*(5q31-33), encodes a nucleolar protein called treacle. The hypothesis for clinical manifestation is haploinsufficiency of treacle during embryonic development. TCS has high inter and intra familial clinical variability, ranging from mild malar hypoplasia to perinatal death due to airway collapse. More than 100 pathogenic mutations were described in *TCOF1* - most of them insertions or deletions causing a premature stop codon and no genotype/phenotype correlation has been observed. We have hypothesized that mutations in the promoter region of the gene in trans with pathogenic mutation can modulate the phenotype. Therefore, the aims of the present work are to characterize the *TCOF1* genes core promoter and to identify mutations in this region that could contribute to the phenotypic variation of this syndrome. We analyzed 1,2 Kb from the *TCOF1* 5' upstream sequence in 25 patients and in 50 normal controls through SSCP and DHPLC, followed by direct sequencing of the identified alterations. We detected 4 polymorphisms: -106AC, -332TC, -948GA, -1025GC. The first two changes were identified only in normal population, both with a frequency lower than 2%; the latter two mutations had a frequency higher than 10% in affected patients and in normal controls, with no difference between the groups. In order to delimitate the minimal promoter region, two fragments, -1200 pb and -600 pb, were cloned in a vector with the luciferase reporter gene. Both were transfected in two human cells lineages, HepG2 and 293T, and were able to promote luciferases gene transcription. The 600 pb vector presented higher activity than 1200 pb vector, indicating that the region between -1200 pb and -600 pb comprises an inhibitor element and that the 600 pb contains the core promoter. We are currently doing functional studies with the regions containing the polymorphisms -948GA, -1025GC as well as with the putative core promoter region. FAPESP/CEPID/CNPq.

A distal regulatory element controls expression of the transcription factor p63. *C. Missero¹, D. Antonini¹, B. Rossi¹, S. Banfi¹, J. Brissette², R. Han², S. Zannini³, T. Di Palma³.* 1) TIGEM, Napoli, Italy; 2) Massachusetts General Hospital, Boston, USA; 3) Federico II University, Napoli, Italy.

The transcription factor p63, a member of the p53 family, is essential for the development of various ectodermal structures, including the epidermis and the hair follicle. Specific mutations of the p63 gene cause five distinct human malformation syndromes that are characterized primarily by limb abnormalities, ectodermal dysplasia, facial cleft, and absence of mammary glands. p63 is a complex gene encoding at least six isoforms with two main transcription start sites, TA and DeltaN, and is primarily expressed in epithelia including the proliferative compartments of the skin. Given its crucial role in skin development and its relevance in some epithelia abnormalities, we set up to identify regulatory genomic regions responsible for the specific expression of the p63 gene in skin. As an initial effort, we cloned and tested the activity of 4kb genomic portions upstream the transcription start site of the TA and DeltaN isoforms. Although these regions displayed promoter activity, comparable activity was obtained in mouse primary keratinocytes as well as in non-expressing cell types. We thus carried out a systematic genomic sequence comparison between human and mouse to search for conserved regions that would act as a skin-specific enhancer for p63. We selected and tested conserved sequences in the p63 genomic locus of at least 100bp in length and 75% identical with a low probability (>1/1000) to be conserved by chance. Out of forty conserved sequences tested four displayed strong enhancer activity. Interestingly, one of them conferred keratinocyte specific expression to a reporter gene, when placed in front of a TK minimal promoter or in front of the DeltaNp63 promoter. The ability of this sequence to recapitulate p63 gene expression was tested by generating transgenic mice, in which the genomic sequence was placed in front of a globin promoter driving LacZ. Functional characterization of the enhancer by mutation analysis, chromatin immunoprecipitation, and siRNA for specific transcription factors binding to it, revealed a previously unsuspected regulation of the p63 gene.

Identification of the regulatory elements controlling expression of the *Chx10* human microphthalmia gene in the retina. L. Weaving, H. Kim, A. Sertie, J. Horsford, R. Abboud, R.R. McInnes. Programs in Developmental Biology and Genetics, The Hospital for Sick Children, Toronto, ON, Canada.

Chx10 is a highly conserved homeodomain transcription factor required for normal eye development. *Chx10* loss-of-function mutations in both human and mouse result in blindness and microphthalmia. *Chx10* is expressed in both retinal progenitor cells and adult bipolar interneurons, the major interneurons of the retina. In the *Chx10^{or-J/or-J}* mutant mouse, there is a substantial decrease in neuroretinal progenitor cell proliferation, and a specific lack of bipolar cells. To understand the role of *Chx10* in eye development, we have begun to identify the enhancers that regulate *Chx10* expression. We have characterised a microphthalmia mouse model designated *Chx10^{or-2J/or-2J}* which has no retinal expression of *Chx10* due to an inversion of ~750 kb. The 5 breakpoint occurs ~18 kb upstream of the *Chx10* start site, disrupting a conserved 2 kb putative regulatory element. To test if this region encompasses a *Chx10* retinal enhancer, we linked the wild-type 2 kb fragment to the 5.3 kb *Chx10* promoter region and a lac Z reporter in transgenic mice. Although expression was observed in adult bipolar cells, there was no expression in retinal progenitor cells. These results indicated that an element within this 7.3 kb fragment is necessary for *Chx10* expression in mature bipolar cells, but not in neuroretinal progenitor cells. To identify other *Chx10* enhancers, we performed sequence alignments among human, mouse and pufferfish, and identified five elements 29 to 50 kb upstream of murine *Chx10*, that are conserved across all three species. We are using both a candidate approach, taking the conserved elements as putative enhancers, and constructing a deletion series of a *Chx10* BAC, in an attempt to identify the progenitor enhancer/s. Identification of these enhancers is the first step in dissecting the regulatory cascade of genes controlling *Chx10* expression.

Identification of the tissue-specific enhancer element responsible for mouse type X collagen gene expression *in vivo*. Q. Zheng¹, B. Keller¹, G. Zhou¹, D. Napierala^{1,2}, Y. Chen¹, A. Parker³, B. Lee^{1,2}. 1) Mole. & Hum. Genetics, Baylor Coll. Med., Houston, TX; 2) Howard Hughes Med. Inst; 3) Respiratory and Inflammation Res. Area, AstraZeneca, Cheshire U.K.

Type X collagen (*Col10a1*) gene is specifically expressed in hypertrophic chondrocytes during endochondral ossification. Its deficiency in human causes Schmid metaphyseal chondrodysplasia (SMCD). Multiple regulatory elements within human, murine and chicken *Col10a1* promoter regions have been reported to direct *Col10a1* expression at different levels by *in vitro* transfection studies. Our previous studies showed that 4kb *Col10a1* promoter containing Runx2 elements can specifically drive weak reporter gene (*LacZ*) expression in the lower hypertrophic zone of transgenic mice. However, *cis* elements directing its high level hypertrophic chondrocyte-specific expression *in vivo* have not yet been described. In this study, we report identification of the tissue-specific enhancer element responsible for high level mouse *Col10a1* expression by generating additional transgenic mouse lines harboring various *Col10a1* promoter and intronic fragments driving *LacZ* as reporter. An 8kb *Col10a1* regulatory region encompassing the same 4kb promoter and second intron can direct reporter gene expression throughout the hypertrophy zone, albeit at low level. In another transgenic mouse line containing a 10kb regulatory element, high level tissue-specific expression throughout the hypertrophic zone was observed. These *in vivo* data suggest the presence of enhancer elements in both *Col10a1* distal promoter and second intron that cooperate with the Runx2 binding elements in the proximal promoter to specify its tissue-specific expression. *In silico* cross species analysis of *Col10a1* shows highly conserved elements within both the *Col10a1* distal promoter and second intron. These elements contain putative AP-1 and Cdx binding sites respectively. We found that transgenic mice using the 6kb and 4.6kb *Col10a1* element could direct *LacZ* expression within the hypertrophic zone. This suggests that the conserved *Col10a1* distal promoter element (-4.6 to -3.9kb) acts as a critical tissue-specific enhancer for *Col10a1* (reporter) expression *in vivo*.

Identification of an evolutionary conserved, putative intronic splicing enhancer in the CFTR gene: a general 5splice site recognition mechanism? *I. Aznarez^{1,2}, G. Deng¹, J. Parkinson^{1,2}, J. Cheung¹, J. Zielenski¹, B.J. Blencowe², L.-C. Tsui^{1,2,3}*. 1) Genetics and Genomic Biology, Hospital for Sick Children, Toronto, Canada; 2) University of Toronto, Canada; 3) University of Hong Kong.

Cystic fibrosis is an autosomal, recessive disease caused by mutations in the cystic fibrosis transmembrane-conductance regulator (CFTR) gene. Proper splicing of the CFTR gene requires accurate selection of splice sites (ss) promoted by elements that are bound by splicing factors. In order to identify intronic splicing elements we performed multi-species alignments of CFTR genomic sequences. Conserved regions downstream of 5ss, spanning 20-40 bp, and located in 12 of the 26 CFTR introns, were considered as candidate splicing elements. We screened for motifs among the conserved sequences by scanning for random hexamer. The scanning analysis identified 2 over-represented motifs present in 9 of the 12 conserved human CFTR 5ss-adjacent intronic sequences. Conversely, the motifs were found in only 2 of the 14 non-conserved sequences ($p=0.004$). Neither motif was found downstream of pseudo 5ss ($n=76$) in the CFTR gene suggesting they could be intronic splicing enhancers (ISE). To assess whether the putative ISEs (pISEs) were present in other genes, we scanned 26,555 protein-coding, intron-containing genes. Results showed that 6,000 genes contained one of the pISEs within 30 bp downstream of the 5ss (3-fold increase over a control screen based on intronic nucleotide content, $p=0.015$). This pISE has a similar consensus sequence to the binding site for TIA-1, a splicing factor known to aid the recognition of 5ss. TIA-1s function is evolutionary conserved which agrees with the identification of the pISE in conserved regions. We are assessing whether TIA-1 mediates recognition of CFTR 5ss through the pISE. Phylogenetic and conservation analyses are being performed for the other pISE-containing 6000 genes. We are also assessing whether the pISE is more often present downstream of constitutive or alternative exons and the optimal distance of the pISE from 5ss to promote splicing. These results will provide insights into the mechanism of 5ss recognition in CFTR and other genes.

Distribution and characterization of lamin A/C mutations: a role for silent variations? *S. Benedetti¹, I. Menditto¹, C. Rodolico², P. Carrera³, D. Toniolo⁴, M. Ferrari^{1,3}*. 1) Dept Molecular Biology, Diagnostica e Ricerca HSR, Milano, Italy; 2) Neuroscience Department, University of Messina, Messina, Italy; 3) Unit of Genomics for Human Disease Diagnosis, IRCCS San Raffaele, Milano, Italy; 4) DIBIT, IRCCS San Raffaele, Milano, Italy.

Lamin A/C mutations have been associated to a variety of pathologies affecting mainly muscular, nervous and adipous tissues. More than 100 mutations scattered along the gene have been described to date and, although no clear genotype-phenotype correlation could be established, some clustering has been recognized. The analysis of 110 patients with different lamin-associated disorders highlighted 14 mutations, 9 of which had not been previously described. The distribution of these variations, mostly missense substitutions, confirms preferential clustering of muscular disorders-associated mutations in exons 4, 6 and 7. In addition, three conservative substitutions were identified and analysed at the RNA level: c.357C>T (R119R) in exon 2, c.1146C>T (G382G) in exon 6 and c.1551G>A (Q517Q) in exon 9. The importance of exonic splicing enhancers and silencers as modulators of the splicing process is increasingly recognised and softwares have been designed to predict gain or loss of interactions with splicing regulatory proteins (serine/arginine-rich, SR). The analysis performed with the ESEfinder software (<http://exon.cshl.org/ESE/>) predicted an increase of the affinity for two SR proteins in the case of the exon 6 substitution, a decrease for the exon 9 variant and no difference for the exon 2 one. mRNA analysis by RT-PCR and sequencing of the c.1146C>T variant revealed the activation of a cryptic exonic splice site leading to deletion of the last 13 bp of exon 6 and consequent frameshift, while no obvious change was observed in the other two variants. These observations suggest that particular attention should be paid when evaluating the pathogenicity of apparently silent substitutions. The use of prediction softwares, although providing useful hints, needs always to be integrated by experimental data.

The effects of temperature and other variables on illegitimate splicing in leukocyte-derived mRNA. A.

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The central dogma of molecular biology posits a flow of genetic information from DNA to RNA to protein. mRNA splicing is central to this process and involves, in eukaryotic cells, a spliceosome composed of upwards of 300 distinct proteins and multiple untranslated RNAs. The specificity of this intricate macromolecular splicing complex is conferred by the interactions of acceptor, donor, and branch site sequences as well as exon splice enhancer elements, with the components of the spliceosome. Errors of splicing, termed illegitimate splicing, can and do occur. These typically involve the insertion or deletion of exons and/or introns which may lead to abnormal transcripts giving rise to truncated or irregular proteins. The inaccurate processing of RNA transcripts may interfere with the diagnostic detection of gene mutations, especially when using approaches such as the protein truncation test (PTT). We have used a model system involving the illegitimate insertion of exon20a into the BRCA2 gene transcript derived from peripheral blood leukocytes (PBLs) to test the effect of a series of variables that may alter the fidelity of splicing. These variables include, aging of the sample, temperature variation, or the introduction of a protein synthesis inhibitor or a mitogen. We have shown that PBL-derived mRNA from a freshly drawn blood sample, even when held at room temperature for a relatively short time, could exhibit illegitimate spliceforms, and that this problem increases with sample aging. Maintenance of stored samples in a heated environment (37C) immediately following venepuncture reduces the occurrence of illegitimate splicing. Culturing PBLs from samples stored at room temperature (25C) for up to 72 hours also resulted in a marked reduction in the proportion of the aberrant isoform. The presence or absence of phytohemagglutinin (PHA) did not appear to affect this process. It was concluded that an effective approach to reducing the problem of illegitimate spliceforms in shipped blood samples was to routinely culture PBLs derived from these samples prior to RNA isolation.

Exon-identity determinants and splicing-based therapeutic strategies in spinal muscular atrophy. *A.R. Krainer¹, L. Cartegni^{1,2}, M. Hastings¹*. 1) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; 2) Memorial Sloan Kettering Cancer Center, New York, NY.

Spinal muscular atrophy (SMA) is a severe autosomal recessive neurodegenerative disorder caused by mutations in *SMN1*. An *SMN1* paralog, *SMN2*, expresses enough functional SMN protein for viability, but not enough to prevent degeneration of spinal-cord motor neurons. *SMN1* and *SMN2* differ by a translationally silent C to T transition at position +6 of exon 7, which causes predominantly skipping of this exon in spliced *SMN2* transcripts in all cells, producing mostly inactive SMN protein. A low level of *SMN2* exon 7 inclusion gives fully functional protein. If inclusion of *SMN2* exon 7 could be improved, the resulting elevation of SMN protein levels may prevent motor neuron degeneration and SMA. We have previously shown that an SF2/ASF-dependent exonic splicing enhancer (ESE) in exon 7 is inactivated by the C to T transition, causing exon skipping. It has been suggested that this transition might instead create an hnRNP-A1-dependent exonic splicing silencer element. We are systematically addressing whether C6T is a loss-of-function or gain-of-function mutation. Our results support the ESE loss-of-function model, and demonstrate that the effect of hnRNP A/B proteins is independent of the C to T transition and reflects their general inhibitory effect on inclusion of alternative exons. These findings are relevant for SMA therapy development, and have mechanistic implications for exon skipping due to exonic mutations, which are common in many genetic diseases. We are pursuing a rational-design therapeutic strategy involving synthetic chimeric antisense/peptide molecules that promote exon inclusion. In addition, small molecules that promote *SMN2* exon 7 inclusion in cultured cells were previously identified, but their mechanism of action remains unclear. We are using a cell-free splicing assay that recapitulates the difference between *SMN1* and *SMN2*, to determine whether these compounds act directly on the splicing machinery, and if so, to determine their precise mechanism of action and specificity.

Up-regulation of CRELD2 expression by a heart defect-associated mutation that interferes with unproductive splicing. C.L. Maslen, D. Babcock, M.A. Jones, B. Ross. Oregon Health & Science University, Portland, OR.

Regulation of protein expression at the level of transcription via unproductive splicing coupled with nonsense-mediated mRNA decay (NMD) is an underappreciated mechanism by which protein abundance can be reduced. Unproductive splicing occurs through alternative exon usage that shifts the reading frame and introduces a premature termination codon (PTC). NMD is triggered when a PTC is detected by mRNA quality surveillance machinery. This requires a pilot round of transcription, hence the acronym RUST (Regulation by Unproductive Splicing and Translation). Gene expression data for *CRELD2*, a candidate gene for cardiac atrioventricular septal defects (AVSD), shows that skipping of exon 2 results in the introduction of a PTC, producing a mRNA species that is subject to NMD. This indicates that *CRELD2* expression is regulated by RUST via exon 2 skipping. In studying the potential association between *CRELD2* mutations and AVSD, we identified a heterozygous 3 bp insertion (132insTTC) at the 5' donor splice site of intron 1 in DNA from a subject with sporadic isolated AVSD. The insertion was not detected in over 400 race-relevant normal chromosomes indicating that this is a disease-associated mutation. The insertion is at the +3 position of intron 1, disrupting the 5' donor splice site consensus sequence. *In vivo* splicing assays were used to determine the consequences of the mutation. As expected, two species of RNA were transcribed from the wild type construct, one with exons 1, 2 and 3 appropriately spliced, the second transcript with exon 2 skipped. However, the insertion mutation construct produced only appropriately spliced product, and did not make transcripts that skip exon 2. Hence, the mutation up-regulates *CRELD2* expression by interfering with exon 2-mediated RUST. This is consistent with our premise that *CRELD2* is a competitive inhibitor of *CRELD1*. Our previous work showed that dominant negative mutations of *CRELD1* are associated with AVSD. Up-regulation of *CRELD2* expression by this mutation may ultimately interfere with *CRELD1* function, thereby increasing susceptibility to AVSD. This defines a new disease mechanism whereby a mutation interferes with RUST.

A new alternative splice variant in BRCA1 generating an additional inframe exon. *A-M. Moisan^{1,2}, J. Fortin^{1,2}, Y. Labrie¹, F. Durocher¹, INHERIT BRCA^s¹, J. Simard¹.* 1) Cancer Genomics Lab, CHUL Research Ctr, Quebec, PQ, Canada; 2) Authors contributed equally to this work and should be considered as equal first author.

Germline mutations in the tumour suppressor gene BRCA1 predispose individuals mainly to breast and ovarian cancer. The BRCA1 gene consists of 24 exons encoding a protein of 1863 amino acids interacting with multiple protein complexes involved in several cellular mechanisms, such as DNA repair, transcription, homologous recombination and cell cycle regulation. Several known BRCA1 splice variants changing the coding region have been reported. They are caused by a series of relatively large deletions as well as the addition/deletion of one or two amino acids due to a usage of leaky splice sites and their regulation and possible cellular function(s) remains under investigation. We report for the first time a novel BRCA1 splice variant caused by an inframe insertion. This transcript, designated exon 13A, is generated by an insertion of 66 nucleotides between exon 13 and 14, due to an alternative splicing of parts of intron 13 (IVS13-2786_-2720). No sequence variants has been detected by direct genomic sequencing of exon13A in 760 individuals originating from 225 French Canadian high risk breast/ovarian cancer families. The exon 13A transcript was detectable in twelve tested total RNA samples from normal tissues and from both ER+ and ER_ breast cancer cell lines as well as several other cancer cell lines. As revealed by real-time PCR analysis, the abundancy of the exon 13A transcript correspond to 20 to 25 % of the total BRCA1 mRNA expression levels in fresh leukocytes, brain and cerebellum tissues, whereas its relative level of expression is about 5% in other samples. It is of interest to note that this novel alternative transcript adds 22 amino acids after residue 1452, thus modifying the primary structure of the trans-activation domain 1 (AD1) as well as the protein-protein interacting domain of BRCA1 with BRCA2, the nuclear androgen receptor, the transcriptional co-activator p300/CBP and the mismatch repair protein hMSH2.

Improving exon inclusion in a human splicing disease: cis acting sequences and the effect of kinetin. S.

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Familial dysautonomia (FD) is a progressive degenerative disorder of the sensory and autonomic nervous system. The major FD mutation is at base pair 6 of intron 20 (IVS20+6 T>C) in the *IKBKAP* gene. This mutation results in a decrease in splicing efficiency that leads to variable, tissue-specific skipping of exon 20. We have developed an *in vivo* minigene splicing system that models the aberrant splicing in FD. Additionally, we have found that the plant cytokinin kinetin dramatically increases exon 20 inclusion in both FD patient cell lines and in our *IKBKAP* minigene system, and this small molecule is currently being evaluated as a potential therapy for FD. The effect of kinetin on *IKBKAP* exon 20 inclusion is at least partially sequence specific since we see no increase in exon inclusion in other genes tested to date. In this study, we aimed to determine the sequence elements involved in *IKBKAP* exon 20 skipping and to identify the specific sequences that are crucial for the action of kinetin. In our *IKBKAP* minigene model, we created a series of sequence changes to cis-acting elements within the exon, within the intronic sequences, and to the 3' and 5' splice sites. We identified numerous sequence changes that improve, reduce or completely disrupt exon 20 inclusion. We then tested all of the altered minigene sequences following kinetin treatment to determine if any sequence changes disrupt or enhance the action of kinetin. We found that with the exception of those sequence alterations that completely disrupt splicing, kinetin had the same overall effect of improving exon inclusion. In order to further investigate the mechanism by which kinetin alters splicing in human cells, we are using microarray studies and testing treated FD cell lines for changes in gene expression. Our findings using the minigene system provide valuable insight into the cis-acting sequence elements required for accurate mRNA splicing. Further, our microarray studies will likely yield downstream targets of kinetin, which may provide additional routes toward therapy for this devastating disease.

Silencing of *MeCP2* in adult mice through RNA interference. *T. Campos-Pereira*¹, *I.G. Maia*², *M. Grippo*¹, *R. Gilioli*³, *I. Lopes-Cendes*¹. 1) Dpto of Medical Genetics, State University of Campinas, Campinas, São Paulo, Brasil; 2) Dpto of Genetics, IBB, Unesp - Botucatu, São Paulo, Brasil; 3) Centro de Bioterismo, Unicamp - Campinas, São Paulo, Brasil.

RNA interference (RNAi) is a recent technology that promotes potent and specific gene silencing through double-stranded RNA molecules such as 21bp small interfering RNAs (siRNAs). This technique has recently been applied to adult mice with success. Rett syndrome (RS) is a neurodevelopmental disorder that affects 1/15000 women, characterized by ataxia, autistic behavior, loss of language skills and stereotyped hand movements. Most cases of RS is associated with mutations in the gene encoding Methyl-CpG-binding protein (*MeCP2*).

We used RNAi in adult mice in order to silence *MeCP2* *in vivo*. For this purpose, we synthesized siRNAs *in vitro* and injected in the tail vein (2 g of siRNA per mouse with PBS). Control groups were injected with either PBS only, irrelevant siRNA or mutated siRNA. Forty-eight hours latter animals were sacrificed and protein samples from liver, kidney, lung, spleen, heart and brain were extracted for analyses. Animals were treated according to the Ethics Commission in Animal Experimentation guidelines for animal care of State University at Campinas.

Western blot analyses confirmed successful knocking down of *MeCP2* in mice kidneys. These animals showed no classical stereotyped forelimb movements, evidencing a brain-selective disfunction of *MeCP2* as the primary cause of RS.

There are three animal models for RS, all of them originated from genomic DNA modification and resembling RS features. Here we report the first animal model using *MeCP2* disruption by RNAi, and the only one with selective silenced of *MeCP2* in visceral organs, but not in the brain. Such approach may shed some light on *MeCP2* function on visceral organs and its contribution to RS.

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Identification of interactions of the Shwachman-Diamond syndrome protein (SBDS). *D.W. Ellenor*^{1,2}, *J.M. Rommens*^{1,2}. 1) Department of Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, Canada; 2) Program in Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, Ontario, Canada.

Shwachman-Diamond syndrome (SDS) is an autosomal recessive disorder characterized by exocrine pancreatic insufficiency, hematological dysfunction and skeletal abnormalities. Mutations in the *SBDS* gene have been associated with SDS. The gene product, SBDS, is a member of a highly conserved protein family of unknown function, with orthologs identified in eukaryotes and archaea. Indirect evidence suggests that the homologs may be involved in RNA metabolism or ribosome biogenesis. In order to investigate the role of the SBDS in mammalian cells, approaches aimed toward the identification of molecules that interact with SBDS were attempted. Exhaustive screens with yeast two-hybrid methodology, using human SBDS and cDNA libraries prepared from human pancreas, bone marrow or liver RNA, did not reveal any direct interactors. Methods that detect only strong binary interactions may not provide suitable approaches, as SBDS may be involved in transient or multi-protein or RNA/protein complexes. Modified interaction detection schemes have been devised using human fibroblasts to identify both protein and nucleic acid interactors. Gentle lysis and mild denaturing conditions are being used for the generation of extracts, together with mass spectrometry techniques (for proteins) or PCR-based recovery schemes (for nucleic acids). To date, SBDS-specific interacting protein bands have been detected and reproduced on Coomassie-stained polyacrylamide gels. The identification of the obtained interactors will provide insight into the function of SBDS and aid in the elucidation of the nature of any associated complexes.

Functional analysis of mutations at EGF2 domain of Factor IX gene. *A.R. Jaloma Cruz¹, J. Mantilla¹, N. Enjolras², J.L. Plantier², C. Vinciguerra³, C. Negrier².* 1) Division de Genetica, CIBO-IMMS, Guadalajara, Mexico; 2) Faculte du Medicine RTH LAENNEC, Francia; 3) Hopital Edouard Herriot, Francia.

Once a candidate mutation has been identified in patients with hemophilia, it is important to determine the impact of the mutated residues in synthesis and functional activity of factor IX (FIX). We studied three mutations at the same codon of FIX gene (C88G, C88Y and C88F) located in the EGF2 domain, which induced different aminoacid changes and distinct clinical behavior of the hemophilia B patients. **METHODS.** The FIX mutants were cloned in an expression vector following in vitro directed mutagenesis. mRNA expression, intracellular production, intracellular trafficking, and secretion were studied in Cos-1 cell line. Kinetics and half-life were measured by pulse chase and biochemical properties were evaluated from purified products. **RESULTS.** The mRNA expression of the 3 mutants was similar to the wild-type FIX (FIXwt). Conversely, the intracellular ER accumulation and the corresponding decrease in secretion (10-15 fold) were shown for the mutants in comparison with FIXwt. Cystein protease inhibitors did not exhibit a significant effect as compared with previously published results for other cysteine mutations located in EGF domain (C71Y and C109Y). Pulse-chase studies demonstrated an increased concentration in culture supernatant at 21h as compared with FIXwt, although the absolute amount was significantly lower, suggesting a secretion defect. Since these different missense mutations were not shown to significantly affect intracellular trafficking or cellular secretion, the hemophilia B phenotype is likely due to additional differences at the biochemical level. **CONCLUSIONS** This study revealed further aspects of factor IX processing that were correlated with the phenotypic characteristics of hemophilia B patients.

Interaction of the familial Mediterranean fever protein with 14.3.3 in an isoform-specific and phosphorylation-dependent manner regulates its translocation to the nucleus. *I. Jeru¹, S. Papin¹, S. L'Hoste², P. Duquesnoy¹, C. Cazeneuve¹, J. Camonis², S. Amselem¹.* 1) INSERM U.468, Hopital H. Mondor, Creteil, France; 2) INSERM U.528, Institut Curie, Paris, France.

Familial Mediterranean fever (FMF), an inherited disorder characterized by recurrent episodes of fever and serosal inflammation, is associated with mutations in the MEFV gene. The corresponding protein, called pyrin or marenostrin, exists in at least two isoforms, the functions of which are largely unknown: a cytoplasmic full-length isoform (pyrin.fl) and a predominantly nuclear isoform generated by in-frame splicing of exon 2 (pyrin.DEx2), raising the question of the mechanisms underlying such different localization patterns. Here, we have identified two members of the 14.3.3 protein family as pyrin-binding partners. Although pyrin.fl interacts with 14.3.3t and 14.3.3e, pyrin.DEx2 does not. We also show that pyrin.fl is phosphorylated and that this posttranslational modification mediated 14.3.3 binding. As shown by co-immunoprecipitation experiments, this interaction is provided by the domain encoded by MEFV exon 2. Further mapping of the region involved in this interaction identified two unconventional 14.3.3 binding sites located in this domain, with serine 208 and serine 209 (site 1) and serine 242 (site 2) acting as critical residues. Using a S208/S209/S242A pyrin.fl triple mutant or wild-type pyrin.fl in the presence of an inhibitor of 14.3.3:ligand interactions, we show that 14.3.3 binding promotes the cytoplasmic retention of pyrin. Taken together, these data indicate that the phosphorylation- and isoform-dependent interaction of pyrin with 14.3.3, which may modulate so-far unknown functions of pyrin, plays a critical role in regulating the translocation of pyrin to the nucleus.

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Investigation of the subcellular localisation of spartin, mutated in a complicated form of HSP, in neuronal cell lines NSC34 and SH-SY5Y. *H. Patel, A.H. Crosby.* Medical Genetics Unit, St George's Hospital Medical School, London, United Kingdom.

The hereditary spastic paraplegias (HSPs) are a complex group of neurodegenerative disorders characterised by lower limb spasticity and weakness. We have shown that mutations in spartin underlie an autosomal recessive variant of HSP (Troyer syndrome, SPG20) present at high frequency amongst the Old Order Amish. Using a spartin antibody, which has been tested against a panel of human tissues and protein extracts from three cell lines (NSC34, SH-SY5Y and CHO), a single band of ~85kDa was identified on a Western blot. We have performed immunofluorescent staining of endogenous spartin to evaluate its subcellular localisation in the human and mouse neuronal cell lines SH-SY5Y and NSC34, respectively. Green fluorescent protein-tagged spartin shows a pattern of expression that is throughout the cytosol and punctate in NSC34 cells. Endogenous spartin also shows a punctate pattern. However, we often observe intense fluorescence at cell process termini and some immunostaining in the nucleus. The pattern of endogenous spartin in differentiated (retinoic acid) SH-SY5Y cells differs from that in NSC34 cells; it is again nuclear and also found throughout the cytosol, however cells often show an intense region of fluorescence adjacent to the nucleus, and a punctate pattern is also clearly visible in cell processes. The intense fluorescence at the cell termini in NSC34 cells is not replicated in SH-SY5Y cells.

Molecular characterisation of interacting proteins with lamin A encoded by LMNA and disrupted by in the laminopathies. *S. Shackleton¹, D. Smallwood¹, A. Fry², R. Trembath¹*. 1) Dept of Genetics, University of Leicester, Leicester, United Kingdom; 2) Dept of Biochemistry University of Leicester, Leicester, United Kingdom.

In a yeast two-hybrid interaction screen using the C-terminal domain of lamin A as bait, we previously isolated the adipocyte differentiation factor, sterol response element binding protein 1 (SREBP1). In the same screen, we identified murine SUN1 (mSUN1) as a lamin A interacting protein. Software algorithms predict mSUN1 to conform to the typical SUN protein structure with an NTD and a CTD separated by 4 transmembrane domains. Using antibodies raised against the CTD of mSUN1 (residues 1-355) is responsible for lamin binding and NE localisation. To investigate the topology of mSUN1 in the NE, digitonin permeabilisation experiments were performed on NIH 3T3 cells labelled with our anti-mSUN1 antibodies and, surprisingly, these studies suggested that the CTD of mSUN1 lies on the outer face of the NE, exposed to the cytoplasm. These findings were confirmed by immunogold scanning electron microscopy of the outer face of the NE. Together, our data suggest that mSUN1 adopts a novel topology, spanning both membranes of the NE, with the NTD responsible for NE localisation by binding to the nuclear lamina at the inner face of the NE, whilst the CTD lies on the outer face of the NE. Like *C. elegans* SUN-1/matefin, mSUN1 may be involved in microtubule dependent nuclear positioning through nuclear-centrosomal attachment, and identifies a further mechanism by which mutations of LMNA, may contribute to the dystrophic processes observed in the laminopathies.

Molecular and cellular characterization of the Down Syndrome Critical Region Protein 2 (DSCR2). *J. Vesa, Y. Brown, D. Greenfield.* Dept Ped, Medical Genetics, Cedars-Sinai Medical Ctr, Los Angeles, CA.

Down Syndrome (DS) is the most prevalent cause of mental retardation affecting approximately one in 700 newborns. Cytogenetic and clinical studies have demonstrated that DS is caused by complete or partial trisomy of chromosome 21. To elucidate the pathways underlying the abnormalities of the DS brain, we have characterized genes that are associated with cognitive defects of DS. Of these, the DSCR2 gene encodes a novel 288 amino acid protein with 85% homology to the corresponding mouse protein. Computational prediction programs suggest a molecular weight of 32.9 kDa for the human protein with one potential N-glycosylation site at amino acid position 129 and two transmembrane domains at positions 72-90 and 208-243. Near the N-terminus, the polypeptide has a low complexity region encompassing amino acids 17-36, where the mouse protein has one additional amino acid. The lack of a predicted signal peptide suggests that DSCR2 is targeted to a cytoplasmic compartment. To test if these predictions hold true, we analyzed transiently transfected HEK-293 and COS-1 cells, using N- and C-terminally FLAG-tagged cDNA constructs. Our expression studies propose that DSCR2 is synthesized as a 44 kDa precursor protein that is trimmed at the N-terminus resulting in a 42 kDa polypeptide. In vitro mutagenesis assays revealed that the difference between predicted and observed molecular weights is not due to a differential glycosylation status of the protein but is more likely caused by other co-/post-translational modifications. Membrane fractionation and subcellular localization studies of the same cell lines imply that DSCR2 is not associated with membranes but is targeted to the cytoplasmic compartment as a soluble form. To determine the expression pattern of DSCR2 in adult mouse brain, we performed immunohistochemical stainings of cryosections with the DSCR2 specific antibody. The results obtained from these experiments showed positive immunoreactivity mainly in the corpus callosum and brainstem. Future co-localization studies with cell-specific antibodies will reveal the cell types and structures where DSCR2 is expressed in the brain.

Role of N-glycosylation in lysosomal neuraminidase. *D. Wang, E. Bonten, A. d'Azzo.* Department of Genetics and Tumor Cell Biology, St Jude Children's Research Hospital, Memphis, TN.

Mammalian lysosomal neuraminidase (NEU1) is crucial for the maintenance of cellular homeostasis because it is responsible for the removal of terminal sialic acid residues on sialo-glycoconjugates. The interaction with PPCA is essential for its intracellular localization and catalytic activation. Loss of NEU1 function is associated with two lysosomal storage disorders: sialidosis and galactosialidosis (GS). NEU1 contains three (human) or four (mouse) potential N-glycosylation sites. The N-glycosylation sites in the murine Neu1 are: Asn180, Asn337, Asn346 and Asn372. Our previous studies showed that both mammalian and insect cell-expressed recombinant Neu1 occurred in at least two differentially glycosylated forms. Eliminating all 4 N-glycans of Neu1 expressed in insect cells made the enzyme unstable and inactive. To better understand the role of the glycan in Neu1, we generated four mutants (Mut1-4), each carrying an Asn to Asp amino acid substitution at 1 of the 4 N-glycosylation sites. We then assessed the biochemical properties of Mut1-4 by expressing them in insect cells and Neu1 deficient mouse lung fibroblasts. Our data revealed that Mut1 was less stable but retained some residual activity in both expression systems. It could be partially transported to the lysosome in cultured fibroblasts. Mut2 and Mut3 purified from insect cells, albeit recovered in fewer amounts, were catalytically active and appeared less heterogeneous on SDS-PAGE. In contrast, deficient fibroblasts transduced with MSCV-based retroviral vectors expressing either Mut2 or Mut3 had somehow reduced enzyme activities. But the activity of Mut2 was rescued by co-expression of PPCA, while that of Mut3 was not. These data suggest that the Asn346 glycosylation site is likely important for the association of Neu1 with PPCA. So far our results indicate that the glycosylation sites in Neu1 have a synergistic effect on the proteins stability and activity. We are currently verifying this hypothesis and in addition we are testing whether the individual glycosylation mutants are effective in the correction of the phenotype in *Neu1*^{-/-} mice. (Supported in part by NIH grant GM060950).

The Valine274Isoleucine allele does not affect transactivation by IRF6 in a cell culture assay. *S. Kondo*¹, *N. Rorick*², *Z. Abrerra*², *A. Russo*³, *B.C. Schutte*¹. 1) Dept Pediatrics, Univ Iowa, Iowa City, IA; 2) Genetics PhD Program, Univ Iowa, Iowa City, IA; 3) Dept. Physiology and Biophysics, Univ Iowa, Iowa City, IA.

Interferon regulatory factor 6 (IRF6) is a member of the Interferon Regulatory Factor family of transcription factors and is expressed in the fusing palate, tooth buds, skin and external genitalia during development. The IRF6 gene encodes a protein of 467 amino acids (aa) that consists of four domains, DNA binding (aa13~aa113), Proline-rich (aa114~aa225), protein binding (aa226~aa412), and Ser/Thr-rich (aa413~aa467). Recently, we reported that mutations in IRF6 cause two orofacial clefting disorders, Van der Woude and Popliteal Pterygium syndromes (VWS; OMIM 119300 and PPS; OMIM 119500, respectively) and that the SNP resulting in a missense change Valine to Isoleucine (V274I) is significantly associated with isolated cleft lip and palate. However, the function of IRF6 and the potential effect of V274I are unknown. In this study, we performed cell culture transactivation assays to determine the functional domain(s) of IRF6 and to measure the effects of mutations on its activity. We demonstrate that IRF6 is a strong activator of transcription, that the main transactivation domain of IRF6 is located in the Proline-rich domain, that the Ser/Thr-rich domain acts in an autoinhibitory manner, that mutations that cause either VWS or PPS reduce the transactivation function of IRF6, and that the V274I allele has no significant effect. Similar results were obtained in two different epithelial cell lines. These results contribute to the understanding of IRF6 structure/function and etiology of VWS and PPS. They are also consistent with the hypothesis that V274I is not a functional polymorphism, although additional studies are necessary.

ATRX shows sequence-specific DNA binding activity. P. Tang¹, A. Argentaro³, D. Higgs³, M. Familiar¹, J.M. Graves¹, V.R. Harley². 1) Department of Zoology, The University of Melbourne, Victoria, Australia; 2) Human Molecular Genetics, Prince Henry's Institute, Victoria, Australia; 3) Institute of Molecular Medicine, Oxford, UK.

Human *ATRX* mutations lead to a condition known as α -thalassaemia mental retardation X-linked (*ATRX*) syndrome, associated with urogenital abnormalities in 80% of cases, ranging from cryptorchidism to male pseudohermaphroditism [1-2].

While the biochemical role of *ATRX* is unknown, a role in gene regulation via chromatin remodelling is speculated [2]. The *ATRX* protein contains a C2-C2 zinc finger (ZF) adjacent to a plant homeodomain (PHD)-like ZF to which 60% of *ATRX* mutations cluster, highlighting the importance of this domain [2].

The *ATRX* protein binds double-stranded DNA homopolymers *in vitro* through its ZF/PHD domain [3]. Using an EMSA-based random DNA oligonucleotide selection assay [4] we observed that recombinant ZF/PHD domain enriches DNA sequences from the random DNA pool over successive rounds. *ATRX*-selected DNAs have been sequenced and show increased binding affinity when compared to the unselected pool.

Using transfected mammalian cells, we show that *ATRX* represses SV40-mediated transcription of luciferase from the pGL3-Control vector without additional cloned sequences. This repression is dose-dependant and the ZF/PHD domain alone is sufficient for repression. We are currently attempting to identify the DNA elements mediating this repression. Our observations suggest a model for *ATRX* function in gene repression involving binding to regulatory regions of specific genes.

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Progressive Myofibrillar Pathology in a Transgenic Mouse Model of LGMD1A. *S.M. Garvey¹, S.E. Miller², M.A. Pericak-Vance¹, M.A. Hauser¹.* 1) Center for Human Genetics; 2) Department of Pathology, Duke University Medical Center, Durham, NC.

Limb-Girdle Muscular Dystrophy Type 1A (LGMD1A) is an autosomal dominant, progressive disorder with onset in the third decade. Two independent missense mutations in the *myotilin* gene cause LGMD1A. Recently, three additional *myotilin* missense mutations were found in patients with myofibrillar myopathy (MFM), a disease similar to LGMD1A. The amino acid substitutions resulting from these mutations occur at the N-terminus of myotilin, a domain with unknown function. Myotilin protein is expressed in skeletal and cardiac muscle, localizes to the myofiber Z-disc, and interacts with α -actinin, α -filamin, and F-actin.

To explore the effects of the LGMD1A mutations on myotilin and muscle function, we generated transgenic mice expressing the thr57ile *myotilin* mutation specifically in skeletal muscle under the control of the human skeletal actin promoter. The transgene includes the human *myotilin* cDNA and an N-terminal c-myc epitope tag. We have generated five expressing lines, and skeletal muscle from two lines have been characterized by light and electron microscopy. Both lines exhibit a progressive myofibrillar phenotype not seen in littermate controls. Masson trichrome-stained cross-sections and longitudinal sections of quadriceps and tibialis muscle suggest discrete disruption of myofibrils. Analysis of 2-, 4-, 5-, 6-, 7-, and 11-month transgenic mice shows an age-dependent increase in both the size and number of myofibrillar lesions. Ultrastructural analysis of an 11-month old transgenic mouse reveals central nuclei, rimmed vacuoles, Z-disc streaming, and large patches of myofibrillar disarray recapitulating that seen in LGMD1A and MFM patients. Muscle from a control transgenic line expressing the wild-type *myotilin* cDNA displays no histopathology. We have also generated a founder mouse positive for the integration of a *myotilin* transgene containing a deletion that removes the N-terminal 79 amino acids. Grip-strength analysis, muscle morphometry, and immunolocalization of sarcomeric proteins will also be discussed.

Unconventional translation of a human retrotransposon. *J.L. Garcia-Perez, R. Alisch, J. Moran.* Human Genetics, Univ. Michigan Medical School, Ann Arbor, MI.

Long Interspersed Element-1 (LINE-1) is an abundant retrotransposon that comprises ~17% of human DNA. The average human genome contains ~100 retrotransposition-competent LINE-1 elements. These LINE-1s contain two non-overlapping open reading frames (ORF1 and ORF2), which are separated by a 63 nucleotide intergenic sequence that contains two in-frame stop codons. ORF1 encodes a RNA binding protein (ORF1p), whereas ORF2 encodes a protein (ORF2p) with endonuclease and reverse transcriptase activities. Both proteins are required for efficient retrotransposition in cultured human cells; however, how ORF2p is translated has been a long-standing mystery.

We previously showed that the LINE-1 encoded proteins preferentially retrotranspose their encoding transcript by a process termed *cis*-preference. Thus, it is likely that ORF2p expression is tightly regulated. Here, we have used a cultured cell LINE-1 retrotransposition assay in conjunction with molecular biological approaches to determine how ORF2p is translated. Our results demonstrate the following: 1) the intergenic sequence is dispensable for LINE-1 retrotransposition; 2) the putative ORF2 AUG initiation codon can be mutated to any other amino acid without drastically affecting LINE-1 retrotransposition (i.e., the resultant constructs retrotranspose between 10-70% wild type levels); however, when the AUG codon is mutated to a stop codon (TAA, TAG, or TGA), retrotransposition is reduced to 2% wild type levels; 3) the synthesis of a functional ORF1p is not required for ORF2p translation; 4) finally, the putative ORF2 AUG initiation codon in a synthetic mouse LINE-1 element also is dispensable for retrotransposition. Taken together, these data suggest that ORF2p can be synthesized in the absence of an AUG codon and that its translation occurs by an unusual mechanism such as ribosome shunting or is facilitated by an unconventional internal ribosome entry sequence.

The leucine zipper domain of ORF1p is required for L1 retrotransposition. *A.E. Hulme, J.N. Athanikar, J.V. Moran.* Department of Human Genetics, University of Michigan, Ann Arbor, MI.

The non-LTR retrotransposon LINE-1 (L1) comprises ~17% of the human genome. Retrotransposition-competent L1s are composed of a 5' UTR, two non-overlapping open reading frames (ORF1 and ORF2), and a 3' UTR that ends in a poly (A) tail. The exact function of the ORF1 encoded protein (ORF1p) remains unknown; however, ORF1p co-localizes with L1 RNA in cytoplasmic ribonucleoprotein particles, which are proposed retrotransposition intermediates. Residues in the C-terminus of ORF1p are needed for RNA binding, while the N-terminus mediates ORF1p multimer formation. Although the sequence of the amino terminus of ORF1p is poorly conserved, a predicted coiled coil domain is conserved among mammalian L1s. Human ORF1p contains a putative leucine zipper (LZ) in this domain, which lies within the region required for ORF1 multimer formation. As this LZ domain is absent from rat, mouse, and rabbit L1s, it is uncertain whether it is required for retrotransposition.

In order to investigate the necessity of the LZ domain for retrotransposition we mutated the 4 leucine residues of the LZ domain to valine residues. These mutations abolished the ability of L1 to retrotranspose in cultured human cells. We hypothesized that this defect was due to the inability of the LZ mutants to form multimers. To investigate this possibility, we conducted GST pulldown and glutaraldehyde cross-linking assays. Unexpectedly, our data demonstrated that the LZ mutant protein could interact under the same conditions as wild type ORF1p in both assays. These results were confirmed using a yeast two-hybrid screen. In addition, LZ mutant protein was found to localize to RNPs, like wildtype ORF1p. Therefore, we conclude that mutations in the LZ domain abolish retrotransposition, but still allow ORF1-ORF1 protein interaction. These paradoxical findings indicate that multimer formation of ORF1 protein is not sufficient for retrotransposition, and suggest that the LZ domain has another role in retrotransposition.

***In vivo* identification of individual active L1 retrotransposons.** *M.dC. Seleme, H.H. Kazazian, Jr.* Dept Genetics, Univ of Pennsylvania, Philadelphia, PA.

Two basic premises must apply for an autonomous transposon to be active *in vivo* : I) The element must have open reading frames and all its key structural sequences intact, II) The element has to be located in a favorable (permissive) genomic environment so that it can be expressed. Cases of human diseases caused by *de novo* mobilization of a closely related group of L1 retrotransposons (Ta-subgroup) indicate that this is a family of autonomous elements in which at least some members definitely comply with the above-cited premises for mobilization. Analyzing the human genome sequence, we have identified 90 L1 elements complying with the first premise. Our *ex vivo* L1-mobilization assay showed that 49% of them are transposition-competent. Based on these data and on the allelic frequency of these L1s, we estimated that an average human carries between 80 to 100 transposition-competent L1s. Because the cultured cells assay tests the transposition capacity of cloned L1s isolated from their genomic environment, our current estimates of active L1s in the human genome do not necessarily reveal their activity potential in their original genomic location. To assess the validity of these estimates *in vivo*, here we investigate whether these L1 elements, active *ex vivo*, are actually expressed in human tissues. Since L1s move through the obligatory synthesis of a full-length RNA intermediate, the presence of these transcripts in tissues can be used as a molecular indication of L1s sitting in permissive genomic locations and can provide a better estimate of the actual number of active L1s *in vivo*. Taking advantage of available L1 sequence data and using an RNA circularization-RT-PCR technique, we have worked out experimental conditions for the individual detection of full-length elements being expressed in human carcinoma cell lines. For example, we were able to identify a full-length transcript, the 5' and 3' ends of which correspond to those of element A1162431 (active on our retrotransposition assay). We have now a useful experimental tool to identify individual L1 elements expressing transcripts relevant for retrotransposition in cells derived from testicular, ovarian and other human tissues susceptible of L1 mobilization.

L1 retrotransposon-mediated stable gene silencing in human cell line. *N. Yang*¹, *L. Zhang*², *H.H. Kazazian Jr*¹. 1) Dept of Genetics; 2) Center for Research on Reproduction and Women's Health and Abramson Family Cancer Research Institute, University of Pennsylvania, Philadelphia, PA.

RNA interference (RNAi) is an evolutionally conserved mechanism of sequence-specific, post-transcriptional gene silencing initiated by small interfering RNA (siRNA) homologous to the gene being suppressed. It is now established as a general method to silence gene expression in a variety of organisms, including mammals. The short-hairpin RNA (shRNA) structure produced by an RNA polymerase III (polIII) promoter has been reported to generate gene-specific siRNA *in vivo*. Both plasmid and viral based vector systems have been used to deliver shRNA into mammalian cells. However, the current systems suffer from limitations including transient effect, demanding viral handling and/or potential insertional mutations. Here we describe a simple plasmid-based system using human L1 (L1Hs) retrotransposon for stable expression of siRNA in cultured HeLa cells. A ~160bp RNAi cassette containing H1 polIII promoter and shRNA sequence targeting a specific gene was inserted downstream of the *neo*-cassette tagged L1Hs in a plasmid. Transfection of such a plasmid into wild-type HeLa cells and subsequent application of G418 led to selection of cell clones in which the RNAi cassette had been stably integrated into the host genome upon L1Hs retrotransposition. Using this system, the mRNA and protein expression of an exogenous gene, GFP, was reduced by up to ~90% as revealed by real-time RT-PCR, western blot and FACS analysis. In addition, expression of an endogenous gene, GAPDH, was successfully reduced at both the mRNA and protein levels as shown by real-time RT-PCR, western blot and immunohistochemistry. Further characterization indicated that L1Hs retrotransposition occurred within 3-8 days post transfection, most transfected cells had no more than one retrotransposition event occurred, and only one integrated RNAi cassette was sufficient to elicit a strong RNAi effect. Our plasmid-based L1 retrotransposon-mediated RNAi system provides a novel strategy for stable gene silencing in cultured cells in a very easy and efficient manner and may have potential applications for *in vivo* molecular therapy.

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Effect of the CTG expanded repeats on the transcriptome of PC12 neuronal cells. *M.A. Bermudez de Leon, P. Gomez-Islas, P. Velazquez-Bernardino, B. Cisneros.* Department of Genetics and Molecular Biology, CINVESTAV-IPN, 07360 Mexico city, Mexico.

Myotonic dystrophy (DM1) is a multisystemic disorder caused by a CTG repeat expansion within the 3'-untranslated region of the myotonic dystrophy protein kinase (DMPK) gene. Recent studies have demonstrated that the disease mechanism involves a dominant gain-of-function conferred upon mutant transcripts by expanded repeats. Although DM1-associated mental retardation suggests that neuronal functions are disturbed by the expansion mutation, the effect of this alteration in neuronal cells has not been approached. To address this issue, we previously characterized PC12 stable clones expressing the 3'-UTR of the DMPK gene with 5, 30 and 90 CTG repeats. Interestingly, we found that expression of 90 CTG repeats inhibits the PC12 neuronal differentiation. A detail characterization of the effects of CTG expanded repeats on the PC12 cell transcriptome would identify genes associated with the differentiated phenotype that are potential targets of the CTG mutation. For this purpose, wild type and CTG90 PC12 clones were treated with NGF for 6 days and total RNA samples were collected from them. The cDNAs of these clones were labeled and hybridized to a high-density cDNA microarray containing 5 thousand different rat genes. We found approximately 786 genes showing an altered regulation in response to the CTG mutation; from this total number, 73% were down-regulated and 27% up-regulated. Among the affected genes, it could be noticed genes involved in cell proliferation, cell survival, adhesion/motility and neuronal differentiation of PC12 cells. This study is supported by the Muscular Dystrophy Association, Inc. Grant No. MDA-3693.

HUMAN ATAXIN-3 STRUCTURAL ANALYSIS: PRELIMINARY DATA. *M.C.C. Garcia*¹, *C.E. Bendetti*², *C.L.P. Oliveira*³, *P.R. Kuser*⁴, *G. Neshich*⁴, *I.C. Torriani*³, *I. Lopes-Cendes*¹. 1) Med. Genet., UNICAMP/FAC. CIENCIAS MED., CAMPINAS, SP, Brazil; 2) LNLS - CAMPINAS, SP, Brazil; 3) UNICAMP/IFGW - CAMPINAS, SP, Brazil; 4) EMBRAPA/BIOINF. - CAMPINAS, SP, Brazil.

We are applying structural and computational methods to study ataxin-3, a protein that when mutated causes Machado-Joseph disease (MJD). Several neurodegenerative diseases are caused by expansion of a polyQ domain within the respective proteins forming aggregates within neurons, possibly due to misfolding. The physiological and biochemical functions of ataxin-3 remain unknown. In this way the small-angle X-ray scattering (SAXS) technique is a very useful tool since it can be performed with proteins in solution, in conditions close to the native state. Recombinant human ataxin-3 (ATX-3N-Q18) was expressed in *E.coli* and purified by affinity chromatography. SAXS measurements were performed in samples containing 10mg/mL of protein. Search of a structural model was retrieved from the SWISS-PROT/TrEMBL database. We applied BLAST/PSI-BLAST to search for homologous proteins in several sequence databases. The multiple sequence alignment was constructed by CLUSTALW. To predict the secondary structure of the proteins we used the PSIPRED prediction server. An initial model of ataxin-3 was analyzed with Sting Millennium suite. From the SAXS experiments we obtained a value for the molecular weight close to the expected for this protein, increasing the reliability of the data. Also, the analysis indicated an elongated conformation and presence of loose parts in the structure. *Ab initio* calculations allowed the construction of low-resolution 3D models. The secondary structure prediction suggests that the protein mainly consists of α -helices. Further studies are being performed to model the structure of ataxin-3. The development of several technologies now proves it feasible to perform mass screening of proteins and the field of proteomics is rapidly progressing. A comprehensive computational analysis of ataxin-3 together with experimental data from the SAXS experiments can contribute to elucidate important features and may serve as guidelines for future experiments.

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Effect of CAT or AGG interruptions and CpG methylation on nucleosome assembly on the (CAG)_n and (CGG)_n tracts of SCA1 and FRAXA. *K.A. Hagerman¹, D.J. Mulvihill², K. Nichol Edamura¹, C.E. Pearson¹, Y.-H. Wang².* 1) Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, Canada; 2) Robert Wood Johnson Medical School, Piscataway, NJ.

Chromatin packaging and nucleosome assembly can regulate many aspects of DNA metabolism. CAT or AGG interruptions within the (CAG)_n or (CGG)_n tracts of spinocerebellar ataxia type 1 (SCA1) or fragile X mental retardation (FRAXA), respectively, confer increased genetic stability to the repeats. We report the formation of nucleosomes on sequences containing pure and interrupted SCA1 and FRAXA repeats having lengths above the genetic stability threshold ($n > 34$). Increased lengths of pure repeats led to increased and decreased propensities for nucleosome assembly on the SCA1 repeats and FRAXA repeats, respectively. CpG methylation of the CGG repeat further reduced assembly. CAT interruptions in (CAG)_n tracts decreased nucleosome assembly. In contrast AGG interruptions in (CGG)_n tracts did not affect nucleosome assembly by hypoacetylated histones. The latter observation was unaltered by CpG methylation of the repeats. Nucleosome assembly by hyperacetylated histones on interrupted CGG tracts was increased relative to pure tracts and this effect was abolished by CpG methylation. Thus, CAT or AGG interruptions can modulate the ability of (CAG)_n and (CGG)_n tracts to assemble into chromatin, the effect of the AGG interruptions is dependent upon both the methylation status of the DNA and the acetylation status of the histones.

Replication mediated instability of the GAA triplet repeat mutation in Friedreich ataxia. *L.M. Hern¹, R. Sharma¹, M. Gómez¹, S. Shah¹, M.B. Delatycki^{2,3}, L. Pianese⁴, A. Monticelli^{4,5}, B.J.B. Keats⁶, S.I. Bidichandani¹.* 1) Depts. Biochemistry & Molec Biol & Pediatrics, Univ Oklahoma Health Science Center, Oklahoma City, OK; 2) Murdoch Children's Research Institute; 3) Univ of Melbourne, Australia; 4) Dept Molec & Cell Biol & Pathology; 5) IEOS-CNR, Federico II Univ., Naples, Italy; 6) Dept Genetics, Louisiana St. Univ., New Orleans, LA.

Friedreich ataxia is caused by expansion of a GAA triplet repeat sequence in the *FRDA* gene. The triplet repeat is polymorphic and unstable, but the mechanisms underlying its instability are poorly understood. Replication of (GAATTC)_n sequences [9-105 triplets] in plasmids propagated in *E. coli* displayed length- and orientation-dependent instability. In addition to short sequence variation upon replication in both directions, large contractions were frequently seen when the "GAA" strand was the template for lagging strand synthesis. DNA replication was also significantly slower in this orientation. To evaluate the physiological relevance of our findings we analyzed repeats of similar length [8-107 triplets] within human leukocytes *in vivo*. Analysis of 9400 somatic *FRDA* molecules using small-pool PCR revealed a similar mutational spectrum, including large contractions. A (GAA)₃₉ allele, which had previously undergone hyperexpansion during intergenerational transmission, was completely stable in somatic cells, indicating that the threshold lengths, and perhaps the underlying mechanisms of repeat instability, are different in somatic and germ cells. Consistent with the stabilization of premutations by interruptions, somatic instability was abrogated by (GAGGAA)_n hexanucleotide interruptions. Our data demonstrate that the GAA triplet repeat mutation in Friedreich ataxia is destabilized during DNA replication, perhaps during lagging strand synthesis, and indicate that this mutation can be reversed.

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Effect of the CTG expanded repeats on the Tau protein phosphorylation in the PC12 cell line. *O. Hernandez-Hernandez, P. Velazquez-Bernardino, L. Quintero-Mora, P. Gomez-Islas, V. Tapia-Ramirez, B. Cisneros.* Department of Genetics and Molecular Biology, CINVESTAV-IPN, Mexico city, Mexico.

Myotonic dystrophy 1 (DM1) is one of the most prevalent human inherited neuromuscular disorders; classical DM1 onset develops during early adulthood and is characterized primarily by myotonia, muscle weakness and progressive muscle wasting; whereas congenital myotonic dystrophy (CMD), the most severe form of the disorder, is characterized by profound hypotonia, mental retardation and developmental abnormalities. The DM1 mutation has been identified as an expanded CTG trinucleotide repeat tract in the 3'untranslated region (3'UTR) of the DM protein kinase gene (MtPK). Although mental retardation suffered by CMD patients indicates that normal neural functions are disturbed by the CTG expansion, the effect of this alteration on neural cells has been poorly approached. We previously established stable transfectans of PC12 neural cell line expressing the reporter gene CAT alone or fused to the MtPK 3-UTR with 90 CTG repeats (CTG90 clone). We found that CTG90 clone did not response to NGF-induced differentiation and the gene regulation of the late differentiation marker MAP2 was disturbed. Recently, intraneural aggregates of hyperphosphorylated tau protein, a brain microtubule associated protein, have been found in brain areas of DM1 patients and transgenic DM mouse. Additionally, a disruption in the normal expression pattern of tau splicing isoforms has been also observed in those patients. These results suggest that alteration of the expression of tau could account for the mental retardation observed in DM patients, however the mechanism underlying such alteration remains unknown. Hence, taking advantage of our neuronal cell model, we investigated the expression and phosphorylation status of tau protein in the wild type and CTG90 PC12 clones. Our results indicated that levels and phosphorylation stage of the tau protein are altered in the PC12 clone expressing 90 CTG repeats. This study is supported by the Muscular Dystrophy Association, Inc. Grant No. MDA-3693.

***EPM1* VNTR-mediated transcriptional repression reversed by SP3 and an LKLF-Gal4 fusion protein.** L. Hsu, G. Manthey, A. Bailis, T. Krontiris. Divisions of Molecular Medicine and Molecular Biology, Beckman Research Institute of the City of Hope, Duarte, CA.

Expansion mutations of gene-associated variable number of tandem repeats (VNTR) are responsible for several human diseases, including myoclonus epilepsy (*EPM1*). The *EPM1* VNTR contains a 12 bp repeat unit that is 100% G+C; the minisatellite is located about 70 nucleotides upstream of the transcription start site of the cystatin B (*CSTB*) gene. The expanded allele reduces the activity of the *CSTB* promoter in certain cells. For insight into the mechanism underlying VNTR-mediated transcriptional repression, we have generated a knock-in yeast strain that survives only when the reporter gene for cycloheximide sensitivity, *CYH2^s*, is down-regulated. Insertion of the expanded *EPM1* VNTR sequence at the 5' end of the *CYH2^s* promoter inhibited its promoter activity, allowing selection of cycloheximide resistant colonies, whereas insertions of the normal (two-repeat-unit) VNTR allele did not alter promoter function and cycloheximide sensitivity. Using a yeast one-hybrid system, we have identified and characterized two potential human *EPM1* VNTR binding proteins, Sp3 and LKLF, and have shown that they are capable of suppressing minisatellite transcriptional repression. Transcriptional down-regulation of the *CYH2^s* gene in our knock-in strain by an expanded *EPM1* VNTR was reversed by transformation of Sp3 cDNA, indicating that Sp3-*EPM1* VNTR interaction regulates gene transcription. The other binding protein of GC-rich DNA we identified using the one-hybrid screen, LKLF, when fused to a Gal4 activation domain and expressed in the *CYH2^s* knock-in, also suppressed the effect of the expanded VNTR allele. (The LKLF clone we obtained in the one-hybrid screen lacked its native activation domain.) Gel mobility shift analyses indicated the specific binding of both these proteins to the *EPM1* VNTR repeat unit. We are now probing chromatin structure near the *CYH2^s* promoter to determine what effects are conferred by the presence of the expanded minisatellite and how expression of Sp3 and LKLF might affect transcriptional repression by the VNTR.

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Molecular genetic study of CAG repeat instability in Drosophila. *J. Jung, N. Bonini.* Dept Biol, HHMI, Univ Pennsylvania, Philadelphia, PA.

Huntingtons disease (HD) and Machado-Joseph Disease (also known as Spinocerebellar ataxia type 3; MJD/SCA3) are among a class of hereditary neurodegenerative diseases due to an expanded CAG repeat within the open reading frame of the gene. One of the key features of these diseases is the instability of the CAG triplet repeat. Strikingly, CAG repeats tend to expand in successive generations, resulting in earlier and more severe disease phenotypes. Somatic instability may also contribute to the severity of the disease. Therefore, it is of interest to understand the molecular mechanisms of triplet repeat instability, in order to understand both germ line and somatic changes in the repeats. Drosophila models have provided valuable insights into the mechanisms of expanded polyglutamine toxicity, suppression, and candidate therapeutic reagents. We have examined CAG repeat instability in a fly model for polyglutamine disease. Our current data suggest that expanded CAG repeats are unstable in Drosophila, and have a strong tendency for further expansion. Understanding the mechanisms underlying repeat instability may provide new avenues for therapeutics.

PABPN1 overexpression leads to upregulation of genes encoding nuclear proteins that are sequestered in oculopharyngeal muscular dystrophy nuclear inclusions. *A.F. Klein¹, L-P. Corbeil-Girard¹, A.M.J. Sasseville^{1,2}, M.J. Dicaire¹, A. Saint-Denis¹, M. Page¹, G. Karpati³, G.A. Rouleau³, B. Massie⁴, Y. Langelier², B. Brais¹.* 1) neurogenetique, CHUM-Hopital Notre-Dame, Montreal, Quebec, Canada; 2) centre de recherche CHUM-Hopital Notre-Dame, Montreal, Quebec, Canada; 3) McGill Health center, McGill university, Montreal, Quebec, Canada; 4) Institut de recherche en biotechnologie, CNRC, Montreal, Quebec, Canada.

Autosomal dominant oculopharyngeal muscular dystrophy (OPMD) is an adult-onset disease with a worldwide distribution. Intranuclear inclusions (INIs) in skeletal muscle fibers are its pathological hallmark. The dominant OPMD mutations consist in expanded (GCN)₈₋₁₃ stretches in the poly(A) binding protein nuclear 1 gene (PABPN1). The (GCN) repeat codes for a polyaniline tract at the N-terminus of the protein. The INIs are known to contain PABPN1, molecular chaperones, ubiquitin, proteasomal subunits and poly(A)-mRNAs. We describe an adenoviral model of PABPN1 expression that produces INIs in most cells. Microarray analyses using the Affymetrix human HG-U133A GeneChip show that PABPN1 overexpression induces a reproducible change in the expression of 202 genes. The majority of the 103 upregulated genes encodes nuclear proteins, many of which with RNA or DNA binding activities. Immunofluorescence microscopy studies reveal that all tested nuclear proteins encoded by eight upregulated genes colocalize with PABPN1 in the INIs: CUGBP1, SFRS3, FKBP1A, HMG2, HNRPA1, PRC1, S100P and HSP70. Moreover, we show that CUGBP1, SFRS3 and FKBP1A are also present in OPMD muscle INIs. Our results demonstrate that the INIs are more heterogeneous than previously thought. They may play an active role in the pathophysiology in OPMD by influencing the availability of some nuclear proteins.

Decreased palmitoylation of mutant huntingtin leads to abnormal trafficking and cellular toxicity. *A. Yanai¹, R. Kang², A. Mullerad², K. Huang², C.J. Opina¹, B.R. Leavitt¹, A.E-D. El-Husseini², M.R. Hayden².* 1) Centre for Molecular Medicine and Therapeutics, University of British Columbia, Vancouver; 2) Department of Psychiatry, University of British Columbia, Vancouver.

Huntington disease (HD) is a hereditary neurodegenerative disorder characterized by severe motor, cognitive and emotional disturbances and a CAG expansion in the HD gene (*htt*). *Htt* is associated with vesicle membranes and its interactions with proteins involved in endocytic pathways, such as HIP1, suggest that *htt* is involved in intracellular trafficking. Post-translational modification by the fatty acid palmitate is crucial for correct membrane targeting for many proteins, including neurotransmitter receptors and those involved in signaling. The association of *htt* with cellular membranes highlighted the potential that *htt* is also modified with palmitate. This is further strengthened by the fact that *htt* interacts with HIP14, a putative mammalian palmitoyl acyl-transferase (PAT), in a polyQ dependent manner. Here we show that *htt* is indeed palmitoylated at cysteine 206. Furthermore, palmitoylation is modulated by CAG size as demonstrated by a marked decrease in palmitoylation ($p < 0.001$) of mutant *htt*. Decreased palmitoylation is associated with abnormal trafficking of mutant *htt*, leading to intracellular accumulation and aggregation and a marked increase (42%; $p < 0.01$) in intracellular toxicity and cell death. The significant modulation of palmitoylation by the mutation in HD and its subsequent effects on cellular toxicity highlight the potential role of altered palmitoylation in the pathogenesis of HD.

Identification and characterization of natural antisense transcripts (NATs) associated to genes involved in eye development. *G. Alfano¹, C. Vitiello¹, C. Caccioppoli¹, T. Caramico¹, M.J. Szego^{2, 3}, R.R. McInnes^{2, 3}, A. Auricchio¹, S. Banfi¹.* 1) Telethon Institute of Genetics and Medicine (TIGEM), Naples, Italy; 2) The Hospital for Sick Children, Toronto, Canada; 3) Department of Molecular and Medical Genetics; University of Toronto, Toronto, Canada.

Natural antisense transcripts (NATs) represent an intriguing class of genes whose role in controlling gene expression is becoming more and more relevant. We describe the identification of eight novel mouse NATs associated with transcription factors (Pax6, Pax2, Six3, Six6, Otx2, Crx, Rax and Vax2) that play an important role in eye development and function. These newly-identified NATs undergo extensive alternative splicing, overlap with the mature processed mRNAs or with the primary unprocessed transcript of their corresponding sense genes, and are predicted to represent either protein coding or noncoding RNAs. Expression studies, by RT-PCR and RNA in situ hybridization, demonstrate that most of these NATs, similar to their sense counterparts, display a specific or predominant expression in the retina, particularly at postnatal stages. Preliminary studies suggest that the overexpression of one of these NATs, CrxOS (Crx opposite strand), in mouse adult retina is able to significantly decrease the expression levels of the corresponding sense gene, Crx, further suggesting that these transcripts are functionally related to their sense counterparts. This subgroup of NATs may play an important role in the control of the molecular mechanisms underlying eye development and function both in health and disease.

USH1C interacting proteins in the retina. *B.R. Evans¹, J.C. Sowden², C.H. Sung³, M. Bitner-Glindzicz¹*. 1) Clinical and Molecular Genetics Unit, Institute of Child Health, London, United Kingdom; 2) Developmental Biology Unit, Institute of Child Health, London, United Kingdom; 3) Dyson Vision Research Institute, Weill Medical College of Cornell University, New York, USA.

Type 1 Usher Syndrome is the most severe form of a heterogeneous group of autosomal recessive deafness-blindness disorders; manifesting as congenital neurosensory hearing loss, progressive retinal degeneration and vestibular areflexia. 5 genes have been identified for the 7 known USH1 loci, and their protein products are thought to interact in both the auditory hair cells and in the retinal photoreceptor cells. USH1C, a PDZ domain containing protein has multiple isoforms that are known to display differing ultrastructural localisation patterns within the inner ear and retina. It is thought to be involved in the interlinking of cadherin 23 (USH1D) at the tips of the hair cell stereocilia, to the growing actin cytoskeleton and is itself correctly localised through interactions with myosin VIIA (USH1B). In the retina, these interactions are also presumed to occur at the ribbon synapses, the site of colocalisation of the Usher type 1 proteins, yet USH1C shows alternative sites of further expression, separate to myosin VIIA and cadherin 23. We undertook a yeast 2 hybrid study, to examine possible USH1C-protein interactions in the retina. We screened a bovine retinal library with the major isoform of USH1C, resulting in 175 colonies for further investigation. We identified other Usher type 1 proteins as expected, as well as scaffolding proteins and a protein that is enriched in the ribbon synapses. We further characterised the resulting interactions using constructs to the separate PDZ domains.

Secretion analysis of 36 myocilin variants, a protein implicated in primary open-angle glaucoma. *S. Gobeil, V. Raymond.* Molecular Endocrinology and Oncology Research Center, Laval University Hospital (CHUL) Research Center, Quebec City, Qc, Canada.

Primary open-angle glaucoma (POAG) is an ocular disease characterized by an optic neuropathy and a progressive loss of the visual fields. Only two of the seven GLC1 genes have been characterized: the trabecular meshwork-inducible glucocorticoid response (TIGR) gene, also known as myocilin (MYOC), at GLC1A, and optineurin (OPTN) at GLC1E. The MYOC gene encodes a secreted 504 aa polypeptide mainly localized in ocular tissues. More than 45 MYOC glaucoma-causing mutations have been reported and those, that have been investigated, prevented secretion of the polypeptides when transfected in cell lines in culture. To investigate if this was a general feature of the mutant polypeptides, we analyzed the secretion status of 36 variants reported in the literature. Myocilin variants were generated by site-directed mutagenesis of an expression vector encoding the human MYOC cDNA. COS-7 cells were transfected with the WT or mutated MYOC constructs. Myocilin proteins were detected in cells and extracellular media by immunoblotting. Cellular integrity was monitored by trypan blue staining. Out of the 36 variants, 15 were found to be secreted from COS-7 cells. Presence of these MYOC variants in the extracellular media was not caused by cell death. Of the 26 variations tested within the olfactomedin (Olf) domain (aa 246-501) of the protein, 19 remained sequestered intracellularly. All the sequestered proteins were disease-causing variants. Only two of the non-secreted variants were localized outside this domain. The first one, R46X, was a truncated protein. The second one, S502P, was only one aa outside the Olf domain. Our results demonstrate that variations localized near or in the Olf domain lead, in a large proportion, to the intracellular sequestration of myocilin showing that this region plays an important role in the folding and/or oligomerization of the protein. Testing for secretion of novel variants within or close to this highly mutated region will help to decipher if these variations may be classified as disease-causing mutants or polymorphisms.

X-linked Congenital Stationary Night Blindness (CSNB1): Nyctalopin is a cell surface glycoprotein attached by a GPI anchor in human but not in mouse. *E.J. O'Connor¹, T. Wang¹, J. Dalley², N. Bulleid², D. Trump¹*. 1) Academic Unit of Medical Genetics, Centre for Molecular Medicine, University of Manchester, Manchester, UK; 2) School of Biological Sciences, University of Manchester, Manchester, UK.

X-linked Congenital Stationary Night Blindness (CSNB) is a non-progressive eye disorder characterised by defective night vision. Complete CSNB (CSNB1) occurs due to mutations in the gene NYX, which encodes nyctalopin. The no b-wave mouse is a naturally occurring model with a deletion within the mouse *nyx* gene. Human nyctalopin, a novel member of the small leucine-rich repeat proteoglycan (SLRP) family, is predicted to attach to the cell membrane via a glycosylphosphatidylinositol (GPI) anchor. Human and mouse nyctalopin share 86% similarity and much of the variation is at the C-terminus (the GPI anchor signal sequence). We have investigated whether nyctalopin is present on the cell surface and whether the attachment is via a GPI anchor in human and mouse. We made constructs containing a flag tag inserted downstream of the signal peptide for both wild type genes (WT NYX, WT *nyx*) and NYX lacking the C-terminal sequence (NYX NA) and expressed these constructs in COS7 cells. We have demonstrated both human and mouse nyctalopin are present on the cell surface and NYX NA is secreted. Furthermore, treatment of cells with phospholipase D released only human nyctalopin into the cell medium confirming a functional GPI anchor in human only. We investigated this further using *in vitro* transcription/translation in semi permeabilised cells with hydrazine to compete with the lipid moiety during anchor addition. These results confirmed the presence of a GPI anchor in human nyctalopin and the absence of a GPI anchor in mouse. Sequence analysis suggests that chimp nyctalopin also uses a GPI anchor for membrane attachment, however rat nyctalopin is similar to mouse. The C-terminal sequence in mouse is predicted to be transmembrane and we are investigating this further. To our knowledge this is the first example of homologous proteins in different species having different methods of membrane attachment.

Functional analysis of the glaucoma-associated TIGR/myocilin protein: the coiled-coil motif at amino acid 78-105 acts as a nucleation site. V. Raymond, L. Letarte, S. Gobeil, M.-A. Rodrigue. Molecular Endocrinology and Oncology, Laval University Hospital (CHUL) Research Center, Quebec City, QC, Canada.

Mutations in *TIGR/myocilin* are associated with 3-4% of open-angle glaucoma, a disorder characterized by optic nerve degeneration and blindness. The protein forms dimers, migrating at 116-kDa, and higher molecular weight (MW) multimers above 180-kDa. Understanding the mechanisms generating these complexes should help to decipher the still unknown function of the polypeptide. We investigated the role of a putative coiled-coil motif at amino acids (AA) 78 to 105; the leucine-zipper at AA 114 to 183; and 5 cysteine residues, in complex formation. Expression vectors encoding normal or mutated myc-tagged myocilin cDNAs were tested by transfecting COS-7 and human trabecular meshwork cells in culture. Intra- and extra-cellular complexes were separated by non-reducing SDS-PAGE and analyzed by immunoblotting. Deletion of the coiled-coil motif (AA 78-105) severely impaired myocilin multimerization above 180-kDa without affecting its dimerization. In contrast, deletion of the leucine-zipper did not impair multimerization nor dimerization. Single substitution of critical AA in the first coiled-coil motif significantly abrogated the ability of the protein to form stable multimers. Triple substitutions between AA 84-99 abolished high MW complex formation without affecting dimerization. The single C to A substitution at position 185 and double C to A substitution at positions 47 and 61 abolished covalent multimerization above 180-kDa. Simultaneous substitutions of C 47, 61, and 185 resulted only in monomers. Mutation of the 245 and/or 433 C blocked myocilin secretion without altering its oligomerization. Our experiments thus demonstrate that the putative coiled-coil motif between AA 78 to 105 was essential for efficient myocilin multimerization whereas deletion of the leucine-zipper motif had no effect on complex formation. These data suggest that the coiled-coil motif at residues 78 to 105 may act as a nucleation site (a region involved in specific recognition of oligomerization partners), the oligomers being subsequently stabilized through disulfide bonds involving C 47, 61 and 185.

Studies of X-chromosome inactivation in cows and coast moles. *Z.C. Yen, S. Karalic, C.J. Brown.* Medical Genetics, University of British Columbia, Vancouver, B.C., Canada.

To date, X silencing has not been well studied in mammals other than humans and mice, yet these species show significant differences in the imprinting of inactivation, the number of genes that escape inactivation, and the functional Xist RNA that is critical for initiating X inactivation. To determine if these deviations are unique to humans we have investigated X inactivation in other Eutherian mammals, namely cows and coast moles, which will also serve to unravel functional regions of Xist via comparative sequence analysis. Thus far, the mechanism by which the Xist RNA coats and silences the X is unknown. Xist sequences of cow, vole, mouse, and human are available and we have recently sequenced >5.5 kb coast mole Xist. The addition of full-length coast mole Xist to a multiple species analysis may highlight conserved functional and structural domains of the Xist transcript. Initial results show that the coast mole Xist cDNA lacks sequence similarity to human exon 2 and mouse exon 5, yet contains additional similarity to a cow and human intron. Since insectivores (coast moles) are often considered basal Eutherians, the apparent lack of this additional mole exon in humans, mice and cows may argue for the loss of ancestral gene function during Eutherian evolution. Hence, the study of different species may identify the origin of this unique functional RNA. As only 5% of X-linked genes in mice escape inactivation, compared to 15% in humans, the inactivation status of various X-linked loci in other species will also allow us to evaluate whether incomplete silencing of the X is a unique human phenomenon. We therefore performed methylation analysis on cow and coast mole DNA for FMR1, ARA, ZFX and SMCX. In both species, ZFX and SMCX appear to escape inactivation, whereas ARA and FMR1 are subject to silencing, resembling the pattern seen in humans. On the contrary, all four loci in mice are found to be subject to inactivation. The status of other X loci will provide further insight into this human-mouse discrepancy. These comparative studies will ultimately paint a more complete picture of the evolution of dosage compensation in the Eutherian subclass.

A haplotype of angiotensinogen gene increases its promoter activity in liver cells and is associated with essential hypertension. *S. Jain, Y. Li, S. Patil, A. Kumar.* Pathology, New York Med Col, Valhalla, NY.

Hypertension is a serious risk factor for myocardial infarction, heart failure, vascular disease, stroke, and renal failure. The incidence of hypertension and complications due to hypertension are even greater in the African-American population. Previous studies have suggested that angiotensinogen (AGT) gene locus is linked with human essential hypertension. We have found an A/G polymorphism at -217 of the human AG gene promoter and have shown that frequency of allele A at -217 is significantly increased in the DNA of African-American hypertensive patients. We now show that -217A always occurs with -1074T in the promoter of human AGT gene. In order to understand the physiological role of these polymorphic sites, we have synthesized reporter constructs containing 1.2 Kb of the 5'-flanking region and 36 bp of the first exon of human AGT gene attached to the luciferase gene with -217A:-1074T and -217G:-1074G haplotypes. Transient transfection of these reporter constructs in human liver cells show that haplotype -217A:-1074T has almost two times increased promoter activity as compared to the haplotype -217G:-1074G. Our gel shift assays show that liver enriched transcription factor C/EBP beta and recombinant glucocorticoid receptor (GR) bind more strongly to an oligonucleotide containing -217A as compared to the same oligonucleotide containing -217G. In addition, liver enriched transcription factor HNF-3 beta binds more strongly to an oligonucleotide containing -1074T as compared to -1074G. Thus interaction of transcription factors C/EBP beta, GR and HNF-3 that bind to haplotype -217A:-1074T may be involved in increased expression of AGT gene containing this haplotype. In conclusion, we show that: (a) AGT promoter containing -217A and -1074T binds strongly to GR and liver enriched transcription factors C/EBP-beta and HNF-3 ; (b) reporter constructs containing haplotype -217A: -1074T have increased promoter activity as compared to the same reporter constructs containing haplotype -217G:-1074G. This increased promoter activity of haplotype -217A:-1074T may be responsible for increased expression of AGT gene and hypertension in human subjects containing this haplotype. Supported by grants from NHLBI and Phillip Morris foundation.

A promoter variation in the human perilipin gene decreases transcriptional activity by 5-fold. *N. Kavaslar, R. McPherson, R. Dent.* University of Ottawa Heart Ins, Ottawa, Ontario, Canada.

The adipocyte-specific protein perilipin is located at the surface of intracellular lipid droplets; in the basal state, it protects stored triacylglycerol from hydrolysis by cellular lipases. Perilipin is the most abundant protein kinase A substrate in adipocytes and plays an important role in PKA-mediated lipolysis. Perilipin has been shown to be an adipocyte-specific target gene of PPAR γ . The absence of perilipin in mouse results in leanness and resistance to diet-induced obesity. By SNaPshot and RFLP analyses, we screened more than 1400 human chromosomes in thin (BMI<25%ile for age and sex) and obese (BMI>30kg/m²) cohorts for variation in the promoter of the perilipin gene. We observed small differences between the genotypic frequencies of the two published perilipin promoter SNPs in these two populations. For the wild-type C allele of rs8179046, the thin cohort had a frequency of 0.73 vs. 0.78 in the obese (p=0.0496); for the wild-type C allele of rs8179045, the thin cohort had a frequency of 0.82 vs. 0.85 in the obese (p=0.0710). For the novel 8-bp deletion 958to-965del, the frequency of the deleted allele was 0.08 in the thin and 0.09 in the obese population. To investigate the effect of SNPs in the perilipin promoter region, we constructed luciferase reporter plasmids containing a 1.7-kb upstream region (positions 1728 to +56) of the human perilipin gene with the different alleles of the SNPs and with and without the deletion. These constructs were transiently transfected into the CHO-K1 cell line. Dual luciferase assays showed that only basal levels of luciferase activity were observed with all the constructs tested except for a construct that carries a point mutation A>G at 1215 bp upstream of the transcription start site. This variation was observed to decrease luciferase activity by 5-fold. MatInspector analysis demonstrated that this variation destroys a PPAR/RXR heterodimer binding site. Further studies will elucidate the functional significance of the putative perilipin promoter PPRE identified in this study.

A haplotype of angiotensin-II receptor type -1 increases its promoter activity and is associated with hypertension in Caucasian women. A. Kumar¹, A. Prater¹, M. Guruju¹, G. Venukonda¹, D. Velez², S. Williams². 1) Dept Pathology, New York Medical Col, Valhalla, NY; 2) Department of Genetics, Vanderbilt University, Nashville, TN.

Hypertension is a serious risk factor for myocardial infarction, heart failure, vascular disease, stroke, and renal failure. The renin-angiotensin system plays an important role in the regulation of blood pressure and angiotensin receptor blockers are potent antihypertensive agents. The promoter of the human angiotensin-II receptor type-1 (hAT1 receptor) gene contains at least five single nucleotide polymorphisms (SNPs) which are in linkage disequilibrium. These SNPs are T/A at -777, T/G at -680, A/C at -214, G/C at -213, and A/G at -119. We have shown that variant -680T always occurs with -777T, -214A, -213G, and -119A thus creating two haplotypes (TTAGA and AGCCG). We have studied the role of these haplotypes in hypertension in 218 hypertensive and 195 normotensive Caucasian subjects by examining the SNP at -680. The frequency of nucleoside T at -680 was 0.790 in hypertensive men and 0.793 in normotensive controls, which is not significant. However, the frequency of nucleoside T was 0.822 in hypertensive women and 0.736 in normotensive controls which is statistically significant ($p = 0.036$). In order to analyze the effect of these haplotypes on the promoter activity of hAT1 receptor we have synthesized reporter constructs containing 1.2 Kb of the 5-flanking region and 55 bp of the first exon. Transient transfection of reporter constructs in H295-R cells showed that haplotype TTAGA has approximately five-fold increased promoter activity as compared to the haplotype AGCGG. These experiments suggest that haplotype TTAGA may be increasing the expression of hAT1 receptor in Caucasian women that may lead to hypertension in this population. Supported by grants from NHLBI and Phillip Morris foundation.

A haplotype of angiotensinogen gene increases its promoter activity in adipocytes and is associated with essential hypertension. *Y. Li, S. Patil, S. Jain, A. Kumar.* Pathology, New York Medical College, Valhalla, NY.

Recent studies have suggested that angiotensinogen (AGT) gene locus is linked with human hypertension. AGT gene has an A/G polymorphism at -6, an A/C polymorphism at -20, and an A/G polymorphism at -217 in its promoter. These single nucleotide polymorphisms (SNPs) affect the promoter activity on transient transfection in human liver cells and are associated with human hypertension in different populations. Since AGT gene is also expressed in the adipose tissue and hypertension is more common in obese subjects, we have examined the role of these SNPs on its expression in preadipocytes (3T3L-1) and differentiated adipocytes. Reporter construct pHAGT1.3luc was constructed by attaching 1222 bp of the 5'-flanking region and 36 bp of the first exon of the hAGT gene to the luciferase gene in the basic vector pGL3luc (Promega). This reporter construct contains nucleoside A at -6, nucleoside C at -20, and nucleoside G at -217 positions. We performed site specific mutagenesis to convert nucleosides at each position and nucleotide sequence of the mutagenized construct was confirmed by sequence analysis. These reporter constructs (500 ng) were transiently transfected in preadipocytes, and three and six days differentiated adipocytes at 80% confluency in six well plates using Fugene reagent. Promoter activity was analyzed by luciferase assay after 48 h of transfection. Results of our experiments show that nucleoside A at -6 in the hAGT promoter had 1.9, 2.5, and 3 fold increased promoter activity in pre-adipocytes, three days and six days differentiated adipocytes respectively. Nucleoside C at -20 had 3 fold increased promoter activity in pre-adipocytes and three days differentiated adipocytes, and 3.5 fold increased promoter activity in six days differentiated adipocytes. On the other hand nucleoside A at -217 had 1.5 fold increased promoter activity in pre-adipocytes as well as differentiated adipocytes. In conclusion, we show that reporter constructs containing nucleoside A at -6, C at -20, or A at -217 in the hAGT gene have increased promoter activity in preadipocytes and differentiated adipocytes. Supported by grants from NHLBI and Phillip Morris foundation.

INFOGENE: AN IST PROJECT FOR THE CREATION OF AN INTERACTIVE PLATFORM FOR PERSONAL GENETIC PROFILE CONSTRUCTION AND DECISION SUPPORT. R. DUNLOP¹, C.

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Citizens with genetic concerns are often confronted with information that is difficult to understand. They may be unable to find appropriate answers to their questions. As a result those people who are not necessarily at risk place additional burdens on geneticists services. Other people who could be genuinely at risk from genetic disease can remain unaware of their possible risks and as a consequence they do not visit a specialist when they should. For both categories of people, the lack of information about genetics represents a gap between the modern scientific world of genetic knowledge and the general public. In order to fill this gap, INFOGENE, an IST project funded by the European Commission, is currently under development. The basic aim of the project is to provide general and, to a much greater extent, personalised user-specific genetic information. The INFOGENE system components are: 1) FrontDoor, a public web site providing the general and personalised information to the public, 2) InfoGenie, a personal software component to hold an individuals private information, 3) GeneAdvisor, a state-of-the-art medical decision support component that will produce personalised advice, 4) GeneKiosk, a knowledge database which will hold accredited information on genetic diseases, family factors, genetic testing, predictive genetics, and other genetic data. The genetic information included in the system, will be regularly updated and validated by, or under the supervision, of an editorial board of geneticists and healthcare professionals. At the end of the project, the system will contain the necessary technological components and sufficient medical content for convincing demonstrations.

Program Nr: 1397 from the 2004 ASHG Annual Meeting

Portable Mendelian Inheritance in Man Database: pdaMIM. *D.W. Stockton.* Dept Molec & Human Genetics, Baylor College Medicine, Houston, TX.

The Mendelian Inheritance in Man (MIM) is an indispensable collection of information on inherited disease that is publicly available through the Internet (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>). Although access to this resource is becoming more convenient with increasing availability of computers in clinical settings and wireless networking, there are still many situations in which review of these data is desired but no Internet access is available. To increase the availability of this information a port of the database to a personal data assistant (PDA) was proposed. There are many technical challenges including the limited storage capacity and processor speed of PDAs. To minimize the limited storage capacity issues, several strategies were employed including fragmentation of the reference data and data compression techniques. To improve performance an indexing system was devised for performing searching of the records. An application for the Palm OS, called pdaMIM, was developed for viewing the database and a companion application to convert the format of the database to be compatible with the PDA. The application on the PDA is able to manage and integrate any data of similar structure as the MIM data through the MIM number reference. As an example an application to convert the Frequency of Inherited Disorders Database (<http://archive.uwcm.ac.uk/uwcm/mg/fidd/>) was developed to instantly associate the disease frequency data available with the other information available for a MIM entry. Continued performance and feature enhancements to the pdaMIM application are planned as well as extending the data available.

The Psychological Impact of a Variant of Uncertain Significance Test Result in BRCA1 and BRCA2 Clinical Testing. *B.W. Althaus, G. Ethington, J.L. Blum.* Baylor-Sammons Cancer Center, Baylor University Medical Center, Dallas, TX.

Purpose: Previous research in genetic diagnosis has shown that uncertainty of diagnosis can be uncomfortable and sometimes distressing for patients to experience. The purpose of this study was to determine the psychological impact of a variant of uncertain significance in clinical testing for hereditary breast and ovarian cancer syndrome. **Methods:** Study participants were recruited from a large hereditary cancer risk program in which cancer education, risk assessment, genetic counseling and a discussion of test results were provided. Hereditary cancer risk program participants (118) who received testing results in the following categories: positive for a deleterious mutation (36), negative for a deleterious mutation (46), and variant of uncertain significance (36) completed questionnaires to determine the impact of the test result. Data from the Quality of Life, Enjoyment and Satisfaction Questionnaire, the Hopkins Symptoms Checklist and the Mishel Uncertainty in Illness Scales were compared to measure the impact of an uncertain test result on the participant. **Summary of Results:** Participants diagnosed with a mutation of uncertain significance scored significantly different from the participants who received laboratory analysis of the presence or absence of a deleterious mutation. Quality of life and psychological distress were not significantly different between study participants who received diagnostic results that were positive, negative, or uncertain for a deleterious mutation. Uncertainty cannot be removed from the testing process, but psychological distress and a decrease in quality of life appear to be prevented by effective communication as shown by these results. Genetic counseling that thoroughly educates and prepares the participant for possible test results and their implications can lessen distress caused by positive or uncertain test results.

Program Nr: 1399 from the 2004 ASHG Annual Meeting

Patient Preferences Regarding Recontact by Cancer Genetics Clinicians. *C.A. Griffin, J.E. Axilbund, A.M. Codori, G. Deise, B. May, C. Pendergrass, M. Tillery, J.D. Trimbath, F.M. Giardiello.* Sidney Kimmel Cancer Center at Johns Hopkins University, Baltimore, MD.

Background: Advances in cancer genetics have lead to new opportunities for early disease detection, predictive genetic testing, & potential interventions. Limited information exists of patient preferences concerning methods of recontact to provide updated information. We hypothesized that colon cancer genetics patients wish to be recontacted about advances in medical genetics.

Methods: We mailed information to 495 individuals (including parents of 32 minors) seen at the Colon Cancer Risk Assessment Clinic at Johns Hopkins between 1996-2003. Included were: letter describing advances in gene testing technology & discovery of MSH6 & MYH genes; detailed fact sheets; & a 15-question survey of patient preferences for notification of & potential uses of new information.

Results: As of 6/1/04, 146 surveys (29.5%) were returned; 60 (11.9%) were undeliverable. 88.4% of those surveyed (S) & 90.4% of responders (R) were Caucasian, 48.8% (S) & 48.6% (R) were male, and mean ages were 51.6 (S) & 53.3 (R) yrs. Most answered that an ongoing relationship between genetics providers & patients should continue (77.3%); that it is the primary responsibility of genetics providers to initiate this contact (69.9%); & that contact should occur only when relevant to the patient (40.4%) or when new discoveries are made (34.9%). Most preferred personalized letters as a means of contact (61.0%). Reasons for/against recontact & circumstances in which individuals would pursue additional genetic testing were also tabulated.

Conclusion: People who have sought evaluation in a colon risk assessment clinic want updated information. This has implications for the consultative nonlongitudinal nature of such clinics & suggests patient preferences for personally-tailored information could be labor intensive. Comparison with responses of 357 participants in a colon cancer gene testing study, along with an estimate of cost of recontact, will be reported. (NCI5U24CA78148).

Perceived ovarian cancer risk: No impact of genetic counseling and testing. *K. Kelly¹, G. Ozakinci², H. Leventhal², M. Marvin³, D. Toppmeyer⁴, J. Baran⁴, M. Schwalb⁵.* 1) Univ of KY, Lexington, KY; 2) Rutgers Univ, New Brunswick, NJ; 3) Spectrum Health, MI; 4) CINJ, New Brunswick, NJ; 5) UMDNJ, Newark, NJ.

Understanding perceived risk of ovarian cancer (PROC) is important as PROC has been linked to ovarian cancer screening and bilateral prophylactic oophorectomy. Hence, individuals need accurate PROC to engage in optimal cancer management strategies. However, PROC has been largely neglected in the context of genetic testing for hereditary BRCA1/2 mutations. The current study is the first to: 1) explore new and theoretically-driven ways to conceptualize PROC; 2) examine change in PROC throughout the process of genetic counseling and testing; and 3) examine the differences between PROC and BRCAPRO model estimated ovarian cancer risk in those testing for BRCA1/2 mutations. Participants were 87 Ashkenazi Jewish women with and without a prior cancer at risk for hereditary BRCA1/2 mutations. PROC (percentage risk; projected age of ovarian cancer onset; survival time with ovarian cancer) was assessed at pre-counseling, post-counseling, and post-results. Repeated measures ANCOVAs were conducted to examine change over time and between-group differences (prior cancer and test result) controlling for age and BRCAPRO model estimated risk of having a mutation. Power analysis indicated our sample size was sufficient to detect small effects. No change in any measure of PROC from pre- to post-counseling or from post-counseling to post-result was detected. For those with no personal cancer history (n=43), PROC: percentage risk was compared to BRCAPRO model estimated ovarian cancer risk to determine the accuracy PROC. Accuracy of PROC did not improve from pre-counseling (mean overestimate: 22%) to post-counseling (mean overestimate: 17%) or from post-counseling to post-result (mean overestimate: 14%). In conclusion and in contrast with our studies of perceived risk of breast cancer, neither genetic counseling nor genetic testing influenced PROC. Innovative methods for communication of ovarian cancer risk are needed in order to increase the likelihood that women will engage in appropriate ovarian cancer risk management strategies.

Initial development of an Adult Medical Genetics Program. *A.C. Sturm¹, S.M. Fitzgerald¹, P.K. Binkley², E.A. Sparks², C.F. Wooley², M.T. Scheuner³, C. Eng¹.* 1) Div Hum Genet, Ohio State Univ, Columbus, OH; 2) Div Cardiovasc Med, Ohio State Univ, Columbus, OH; 3) Office of Genomics and Disease Prevention, CDC.

Over the last 6 months, the Ohio State University (OSU) Medical Center Division of Human Genetics formed The Adult Medical Genetics Program. This Program complements OSU's Clinical Cancer Genetics program by offering consultations for all other adult-onset medical genetics conditions, focusing primarily on cardiovascular, pulmonary, and benign hematological genetic conditions initially. Patients are referred for a comprehensive genetics consultation, where they meet with both a genetic counselor and physician as part of a virtual multidisciplinary clinic. In order to inform central Ohio physicians of this unique clinical offering and to facilitate appropriate referral of patients, the following steps were taken. Indications for referral fact sheets were developed for cardiac, pulmonary, and benign hematological genetic conditions and distributed to all faculty and staff within the Departments of Internal Medicine and Family Medicine at OSU and were mailed to 230 cardiologists and pulmonary specialists in Franklin and surrounding counties. The Director and senior adult genetic counselor visited the Divisions of Cardiovascular Medicine, Pulmonary Medicine, and Hem/Onc at OSU in order to educate these physicians about appropriate indications for referral. A Familial Cardiovascular Risk Assessment Tool was developed to screen patients at an OSU Congestive Heart Failure Clinic. This tool incorporates information about specific diagnosis and age at diagnosis on all 1st- and 2nd-degree relatives of the proband; a list of 22 medical conditions associated with cardiovascular disease facilitates ease of use. The completed tool is then used for triage of high risk patients to the Program for a complete genetics consultation. The tool is designed to ascertain families with a high risk of coronary artery disease, familial cardiomyopathies, arrhythmias, aneurysms, clotting disorders, and other genetic cardiovascular conditions. Thus far, a family with long QT syndrome and several families with either dilated cardiomyopathy or high risk coronary artery disease have been evaluated.

Raising awareness of behavior in genetic syndromes and fetal alcohol syndrome in foster care, changes parental framework for discipline. *R.B. Sampilo*¹, *A. Perszyk*². 1) Child Guidance Center, Jacksonville, FL; 2) Pediatric Multispecialty Ctr, Univ Florida, Jacksonville, FL.

Children in foster care may have speech problems, ADHD, history of maternal drug or alcohol abuse, or behavioral genetic syndromes. Information may not be maintained in a child's file. Awareness of the learning style and behavior concerns present in particular syndromes can change the disciplinary styles of house parents and influence long-term outcome, placement and successful rearing. Purpose: To teach awareness of different disorders, individual temperaments, and cognitive limitations to youth care workers, foster parents and case managers to influence individual behavior programs, improve placement and support long-term care plans. Method: Over a ten-year period, efforts to educate the foster parents, counselors, social workers and therapists with lectures and handouts, given to individuals, local or state agencies and programs that serve the foster care system. Additional, short-term programs to raise the knowledge base and better support those in the frontline of providing services for these children - often in areas with high turn-over. Questionnaires were given before and after lectures to assess short-term responses. Long-term follow-up was by phone call or direct face-to-face interview. Significant impact was found in the planned discipline program for the child. Secondary effects, such as school programs that were modified in like manner show carry-through of these disciplinary styles. Short-term indicators were measured in a limited study. Conclusion: Tangible responses could be measured from the social workers and youth care givers that work with disadvantaged youth. Straightforward talk with handouts, Internet website links, and useful tips for those dealing with children is a practical way to improve awareness and influence the treatment options. Continued support to help social services should be looked at regionally. Regional differences may vary but a positive response is expected based on the limited assessments reported.

Counseling the families of two patients with atypical phenotypes following the prenatal diagnosis of Turner syndrome. *C.T. Dvorak¹, C. Techakittiroj¹, H.C. Andersson^{1,2}, G. Pridjian^{1,2,3}, M.M. Li^{1,2}.* 1) Human Genetics Program, Tulane Sch Medicine, New Orleans, LA; 2) Dept of Pediatrics, Tulane Sch Medicine, New Orleans, LA; 3) Dept of OB-GYN, Tulane Sch Medicine, New Orleans, LA.

We counseled the families of two patients born with unexpected phenotypes following a prenatal diagnosis of 45, X (Turner syndrome) by amniocentesis. Patient A presented as a small for gestational age male with minor dysmorphic features (flat nasal bridge, midface hypoplasia, preauricular pits) and normal male genitalia. A peripheral blood chromosome analysis confirmed a karyotype of 45,X at the 650 band level. Subsequent FISH analyses revealed an unbalanced cryptic translocation between the short arm of the Y chromosome (containing the SRY gene) and the long arm of chromosome 15. Patient B was born with ambiguous genitalia (clitoral hypertrophy). A peripheral blood chromosome analysis revealed mosaicism of three cell lines: 45, X (8/25 cells), 46,XY (5/25 cells), and 46, X +mar (12/25 cells). A FISH analysis with X and Y chromosome specific probes demonstrated that the marker had originated from the Y chromosome. These cases illuminate the complexity of prenatal diagnosis and the need in routine prenatal care for referral to genetic specialists, who are able to provide informative, nuanced interpretations of test results. We discuss the clinical and psychosocial challenges faced when interacting with families confronted with unexpected clinical outcomes.

Self-Concept in Carriers of Balanced Chromosomal Rearrangements & the Perceived Impact of Genetic Make-Up on Self-Concept. *A. Thomas^{1,5}, D. Chitayat^{1,2,5}, E. Winsor^{3,5}, A. Kaiser⁴, C. Shuman^{1,5}.* 1) Clinical and Metabolic Genetics, The Hospital for Sick Children; 2) Prenatal Diagnosis and Medical Genetics, Mount Sinai Hospital; 3) Laboratory Medicine & Pathology, Mount Sinai Hospital; 4) Haematology & Oncology, The Hospital for Sick Children; 5) University of Toronto.

Self-concept is defined as how an individual perceives him or herself; some concern has existed in the genetic counselling profession that an individual's self-concept may be negatively impacted by one's genetic carrier status. The purpose of this study was to explore self-concept within a genetic context and had two main aims: 1) to measure the self-concept of carriers of balanced chromosomal rearrangements 2) to determine the perceived importance of genetic make-up on sense of self. The Tennessee Self-Concept Scale (TSCS) was used to measure self-concept. An additional questionnaire, entitled Self-Concept and Genetic Make-up (SCGM) was developed and used to examine the perceived impact of genetic make-up on self-concept. Twenty-six carriers of balanced chromosomal rearrangements and 41 control individuals completed both questionnaires. Results indicated no significant differences between the carriers and controls on the TSCS responses. This suggests that self-concept is not affected by the knowledge that one is a carrier of a balanced chromosomal rearrangement. The results of the SCGM questionnaire indicate that neither carriers nor controls consider their genetic make-up highly important to self-concept. However, carriers and controls varied significantly on their perceptions of the role of genetic make-up with regard to the physical aspect of self-concept; controls perceived the role of genetics as stronger ($p=0.002$). This decreased perception of genetic make-up on physical self-concept in carriers could be due to having been informed that being a carrier would not pose any health-related risks. The reliability of the SCGM scale was adequate but not ideal for clinical research. The data from this study indicate that self-concept is not affected by knowledge of carrier status, and that genetic make-up is not a primary consideration for individuals when they appraise themselves.

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TELEGENETICA: AN ONLINE CONSULTATION SERVICE FOR HEALTH PROFESSIONALS. *M. Del Campo, B. Gener, J. Del Valle, V. Rodriguez, L.A. Prez Jurado.* Genetics Unit. Dept of Experimental Sciences, Universitat Pompeu Fabra, Barcelona, Spain.

Telegenetica (www.telegenetica.org) is an online consultation service in clinical genetics open to registered professionals who seek diagnostic support or specific information for a patient in Spanish. The website contains specific forms to guide non-genetics specialists in the clinical history and examination steps. The consultations are directly replied by board-certified genetics specialists. For those cases without a firm diagnosis, the patients are included in an online discussion board of collaborating specialists, integrated in a Network of Genetics Centers in Spain (www.recgen.net). The project has initiated a collaboration with Teledismorfologia, a similar project based in Chile who serves several Latinamerican countries. The program is also directly linked to Orphanet, the European online encyclopaedia and database of services in rare disorders (www.orpha.net). During the initial 18 months of Telegenetica, 179 health professionals have registered: mostly physicians, but also biologists, pharmacists, teachers, psychologists or nurses. Paediatricians, obstetricians, geneticists and family doctors submitted most consultations. A specific diagnosis has been established for over 50% of cases submitted. A satisfaction questionnaire for participating professionals and patients, and a pilot study for the blind evaluation of the patients referred online is being currently completed. The service is presented to the patient as a confidential and anonymous specialist consultation, but the referring doctor is responsible for providing genetic counselling or referring the patient to a Clinical Genetics service. According to all stakeholders in the project, Telegenetica is providing efficient diagnostic support and accurate genetic counselling to professionals and patients, as well as contributing to education in Genetics of other specialists. Telegenetica is funded by Novartis and Fondo de Investigaciones Sanitarias (FIS): proyecto PI021009 and Red de Centros de Genética Clínica y Molecular (C03/07).

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Comparison of questionnaire and counseling family history information. *J.R. Sprague, W.C. McKinnon, M.E. Wood.* Hematology/Oncology, University of Vermont, Burlington, VT.

Purpose: An accurate cancer family history is an important tool in identifying syndromes and making decisions regarding genetic testing. We sought to assess the accuracy of our family history questionnaire by comparing data obtained in the questionnaire regarding cancer diagnoses to that obtained by the genetic counselor sitting with the patient. **Methods:** From 1995-now more than 700 families have been seen in the Familial Cancer Program Clinic associated with the Vermont Cancer Center. Charts from 100 families were chosen at random for this study. Questionnaire and genetic counselor data regarding individuals diagnosed with cancer and age at diagnosis were entered into a database. 1st and 2nd degree family member information was entered regardless of cancer history. 3rd degree family members were included only if they had cancer. Comparison was made between cancer cases identified by both methods. **Results:** The genetic counselor identified more total family members compared to the questionnaire (1855 vs. 1663) and fewer cancer cases (562 vs. 578). 54% of charts showed no difference between the total numbers of cancer cases reported by either method. 81% of charts had a difference of one or less, reported cancer case between the two methods. Exclusion of 3rd degree relatives increased this to 97%. 92% of charts had a difference of two or less reported cancer cases (98% if 3rd degree relatives excluded). The number of family members reported to have multiple cancers was nearly identical between the questionnaire and genetic counseling sessions (57 and 58 respectively). Closer examination of the data reveals that approximately 15% of the time, the affected individual was different in the questionnaire compared to that identified in the counseling session. **Conclusions:** This family history questionnaire appears to be a relatively accurate tool for obtaining a family history of cancer, future efforts will focus on the accuracy of cases reported (individual identified and pathology). Having a family history prior to genetic counseling sessions will lead to a more productive session and should be routinely done.

The Nowgen network: an on-line system to promote collaboration and information exchange within the human genetics community. *A.C. Rose^{1,2}, W.S. Johnson², G. Wainwright², J. McQuillian², H.R. Middleton-Price¹, D. Donnai¹, R. McDermott¹.* 1) Nowgen, St Mary's Hospital, Manchester, United Kingdom; 2) MerseyBIO, Crown Street, Liverpool, United Kingdom.

Nowgen aims to ensure that developments in human genetics research and thinking are successfully applied and widely understood. However, obstacles to this include difficulties in finding both up-to-date, relevant information and expert, collaborative partners. To address these issues, Nowgen and MerseyBIO have worked together to develop a technologically advanced website. Within the site, called the Nowgen Network, virtual 'communities' are being formed to support inter-institutional and interdisciplinary genetics initiatives, with members of the Nowgen team working closely with communities to support their use of the system. Initially, a number of pilot communities are being setup to focus on clinical, ethical, legal, scientific and educational issues associated with genetics. From a single point of access, users can utilise powerful tools, allowing them to identify partners, obtain expert advice and retrieve personalised, current information. Automatic information retrieval tools use advanced software that can search web pages and many different file types. Community interactions are supported by features including multi-user, real-time conferencing and chat facilities. Importantly, the website can accelerate information retrieval, support collaborations without geographical limitations and facilitate the dissemination of knowledge in support of the genetics community.

Orofacial clefts: Do mothers of children know why it happens? A case study. *M. Obara¹, T. Mosby^{1,2}, H.S. Oh¹, T. Pawar¹, A. Shaw¹, M.M. Tolarova¹.* 1) Craniofacial Genetics, Department of Orthodontics, University of the Pacific School of Dentistry, San Francisco, CA; 2) Pediatric St. Jude Children's Research Hospital 332 N. Lauderdale, Memphis, TN.

Cleft lip and palate are common, serious birth defects whose etiology is still not completely understood. A vast majority of studies are focused on analysis genetic factors, lifestyle, nutrition and other well defined factors. This pilot study explores as clues to the etiology of clefts the knowledge, beliefs, and reports of environmental exposures. All data for this study were collected through personal interviews with the mothers of cleft children during the Rotaplast missions to Guatemala City and Antigua Guatemala during the years 2001-2004. Our population consisted of 448 Guatemalan mothers of children with nonsyndromic cleft lip with or without cleft palate. Out of 449 mothers interviewed, 304 mothers (67.86%) answered the question: Why do you think your child was born with a cleft? The most common response to that question was that they didn't know the cause of the anomaly in their child (36.17%). A most common answer, after excluding those who didn't know, was the eclipse (23.03%). Other responses were: a lack of vitamins and other nutritional factors (12.5%), and heredity (11.19%). Interestingly, same percentage (34 %) of mothers of cleft lip and palate children and mothers of cleft lip children suspected the eclipse to cause a cleft. These findings raise many questions: Why is it such a popular answer? Is this a cultural belief or is there an association through some genetic or environmental factor that we do not know? For example, are mothers with a family history of clefts more likely to have received through many generations, a traditional explanation such as an eclipse? Is this answered confounded by the mothers education? These and other clues will be analyzed, controlling for known factors (low socioeconomic factors, poor nutrition, familial occurrence of a cleft) and will lay down a cultural background for the interpretation of other genetic-environmental causes of clefts. The fieldwork for this study was supported by Rotaplast International Inc.

Educating genetic counsellors in Australia: Developing an international perspective. *M. Aitken*^{1, 4}, *M.A. Young*², *L.J. Sheffield*^{3, 4}, *M. Sahhar*^{3,4}. 1) Genetics Education Unit, Murdoch Childrens Research Institute, Parkville, Victoria, Australia; 2) Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia; 3) Genetic Health Services Victoria, Murdoch Childrens Research Institute, Parkville, Victoria, Australia; 4) Department of Paediatrics, University of Melbourne, Parkville, Victoria, Australia.

The demand for genetic counselling services is increasing worldwide. The development of the education and training process in Australia has been different to those of other countries namely the United States of America (USA), the United Kingdom (UK) and Canada. In Australia the profession of genetic counselling has developed over the last 25 years. Based directly on the American model of working in a team with the clinical geneticist, the training has, due to local geographic, health care system and educational factors, evolved differently. Nevertheless, the core competencies of the profession are similar to those developed in other countries, notably the UK and the USA. A new Australian set of core competencies is proposed. The major difference with the Australian genetic counselling programs is that they are a 1-year graduate diploma course as opposed to a 2-year masters program. One of the reasons for this related to the pressing need for trained genetic counsellors who were then available to complete their clinical training while on the job. This is proposed as a model for genetic counsellor training in other countries, especially developing countries. To this end, there is the potential for an Australian role in training genetic counsellors in South East Asia. Nevertheless, changes to the Australian education system in the past five years and increasing numbers of genetic counselling graduates mean that there is a possibility for the Australian system to consider moving to a 2-year masters program. This would also enable dialogue to continue regarding genetic counselling certification and reciprocity between Australia and other countries.

FAMILY COMMUNICATION OF PROSTATE CANCER RISK: A PILOT STUDY. *M. Alvarado*¹, *K. Siegmund*², *J. Fauror*³. 1) Kaiser Permanente, Pasadena, CA; 2) USC/Norris Comp. Cancer Center, Los Angeles, CA; 3) Genzyme Genetics, Long Beach, CA.

Prostate cancer (PC) is the most common non-cutaneous malignancy in males. Approximately 5% to 10% of PC, and a higher proportion of early onset cases (Bratt, et al, 1999), may be due to dominantly inherited alterations in cancer susceptibility genes. At least half a dozen different loci have been identified in affected families (Nwosu, et al, 2001). Evidence suggests that men with a close relative affected by PC have an increased risk of the disease. Awareness of their increased PC risk and available screening options may lead to earlier diagnosis and increased survival for men with familial risk. We explored factors influencing communication about PC among affected men and their sons and brothers. Two hundred surveys were distributed to a convenient sample of PC clinic patients and support group participants at an NCI-designated cancer center. Forty-four men completed the anonymous 6-page survey that included questions on demographics, personal and family history of cancer, perception of PC heritability and perception of their relatives' PC risk. Participants had a mean age of 69.4 years and most were Caucasian. Two-thirds were recruited from support groups. Thirty-three of the 44 participants had living brothers or sons, and most reported speaking to their relatives "a few" or "many times" since their diagnosis (79% for sons, 69% for brothers). Most participants (73%) believed that PC can run in families and overestimated the proportion of PC that is inherited ("60%-69% of PC is inherited" was the most common answer). Nine (20%) men reported that one of their physicians discussed the PC risks of their relatives, and this proportion was higher for those with a family history of PC. Conclusions: The majority of men in this study communicated with their sons and brothers about PC, regardless of the family history of cancer. Few reported any barriers to communication. Since most participants came from PC support groups our sample may be biased. Educational tools about familial PC risk may help physicians who care for men with PC in counseling their patients regarding family history and PC.

Women's attitudes toward testing for fragile X carrier status: a qualitative analysis. *A.E. Anido¹, L.M. Carlson², L. Taft¹, S.L. Sherman¹.* 1) Department of Human Genetics, Emory University, Atlanta, GA; 2) Rollins School of Public Health, Emory University, Atlanta, GA.

Fragile X syndrome (FXS) is primarily due to a repeat expansion mutation found in the FMR1 X-linked gene. We have conducted a qualitative analysis of responses from women concerning their attitudes toward testing for carrier status of the fragile X mutation among reproductive-age women. We obtained responses from focus groups including women with and without FXS in their families. We found the following themes: 1) the motivation for carrier testing and need for information differed by family history of FXS and parental status, 2) the timing of carrier testing with respect to a woman's life stage dictates whether carrier information will be viewed as beneficial or detrimental and 3) mothers of children with FXS have difficulty formulating their opinions on population screening because of their unique experiences surrounding their own carrier diagnosis and their relationship with their children with FXS. There was evidence that non-carrier women from the general population would be wholly unprepared for positive carrier results. These findings have significant implications for genetic counseling as well as for population screening. Preliminary data of in-depth interviews of carrier women from the general population will be presented.

Expectations and satisfaction with genetic counselling services. *F.P. Bernier¹, S.G. Crawford², B. Sibbald³, R. Kohut⁴, D. Dewey^{2,5}.* 1) Medical Genetics, University of Calgary, Calgary, AB, Canada; 2) Behavioural Research Unit, Alberta Children's Hospital, Calgary, AB, Canada; 3) Alberta Congenital Anomalies Surveillance System, Alberta Children's Hospital, Calgary, AB, Canada; 4) Calgary Health Region, Calgary, AB, Canada; 5) Pediatrics, University of Calgary, Calgary, AB, Canada.

Objective: To explore novel ways of evaluating and improving genetic counselling services by examining the relationship between patients expectations and their satisfaction with the genetic counselling. **Methods:** This study was a prospective cross-sectional survey in which the expectations and satisfaction of patients seen in an outpatient setting through a Medical Genetics clinic were assessed. Pre-counselling expectations were assessed using the questionnaire by Michie et al.(1997), while the post-counselling questionnaire asked what information was provided to patients at counselling, whether their expectations were met, and also included the Satisfaction Inventory developed by Shiloh et al. (1990). Fifty eight patients completed both questionnaires, and 320 completed the post-counselling questionnaire. **Results:** Overall satisfaction with the genetic counselling service was high (3.69/4.00). No significant correlations emerged between client satisfaction and the expectations ratio (# of services hoped for divided by # of services received), or expecting and being offered DNA testing. A higher total # of services, as well as receiving positive information, was significantly correlated with higher satisfaction scores, however, having previous experience with the condition was significantly correlated with lower instrumental, procedural and total satisfaction scores.

Conclusion: The nature of the information received at the counselling session was an important predictor of client satisfaction, as were patient expectations and previous experience with the condition. Without suitable measures or an understanding of the processes that influence patient satisfaction with the genetics counselling service, implementing improvements to the service remains challenging.

Birth Prevalence of Cleft Lip with or without Cleft Palate in Asian Populations: A Meta-Analysis. *M.E. Cooper¹, J.R. Sank², M.L. Marazita¹*. 1) Center for Craniofacial and Dental Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Division of Genetics, Department of Pediatrics, University of Florida.

Cleft lip and cleft palate are among the most common congenital abnormalities. Reported birth prevalences range from 1/1,000 in Caucasians to as high as 2 per 1,000 in Asians. Accurate estimates of birth prevalence rates are necessary for genetic analyses and genetic counseling. The aim of this study is to clarify the birth prevalence of clefts via a meta-analysis of 45 studies involving non-overlapping Asian populations, representing 12,612,166 total pregnancies (TP=live births and pregnancy losses). The table below summarizes the 95% C.I. on the estimated birth prevalences per 1,000 pregnancies for nonsyndromic (NS) cleft lip with or without cleft palate (CL/P), NS plus syndromic (S), total pregnancies (TP), and live births (LB) only. For All Asians as well as Chinese, Japanese and Other subsets, the birth prevalences are significantly greater than the Caucasian rate of 1.0/1,000. Notably, no C.I. includes the often quoted figure of 2.0/1,000 for Asians which appears to be based on early Japanese studies that included large numbers of pregnancy losses and both S and NS cases. From the table, the S+NS TP CL/P Japanese rates are significantly higher than the Chinese or Other subgroups, but if only NS LB are considered, there are no significant differences among the Asian subgroups. These results demonstrate the extreme importance for current population-based studies of clefts to include careful delineation of syndromes, cleft type (CL, CL+P, CP), and birth status (live births versus pregnancy losses). Supported by NIH GRANT #DE09886.

	All Asians	Chinese	Japanese	Other Asians
S+NS TP CL/P	1.33(1.31, 1.35)	1.26(1.23, 1.28)	1.77(1.69, 1.85)	1.41(1.34, 1.48)
NS LB CL/P	1.19(1.13, 1.25)	1.20(1.11, 1.28)	1.18(1.09, 1.28)	1.22(1.04, 1.42)

Title: Perceived personal control as an outcome measure of genetic testing for hearing loss. *M. Fox¹, A. Martinez¹, B. Crandall¹, N. Shapiro¹, M. Telatar¹, Y. Sininger¹, W.W. Grody¹, L.A. Schimmenti², C.G.S. Palmer¹.* 1) Dept Pediatrics & Genetics, Univ California, Los Angeles, Los Angeles, CA; 2) Dept Pediatrics & Genetics, Univ Minnesota, Minneapolis, MN.

Abstract: We have begun a longitudinal prospective study to determine the impact of genetic testing on the EHDI process. Genetic testing for GJB2/GJB6 mutations in infants with hearing loss is offered to parents and results are given through a genetic counseling session. Parents accepting enrollment are given questionnaires to determine parental anxiety and perceived personal control (PPC) to measure the impact of genetic testing at enrollment, 1 month after receiving genetic test results and 6 months later. PPC is divided into three dimensions: cognitive control (the ability to understand genetic test results), behavioral control (the ability to undertake interventions based on the results) and decisional control (the ability to make informed choices that influence a child's future). We present data from 29 parents in 22 families at enrollment and 17 parents in 13 families one month after result disclosure. At enrollment, parents had a mean anxiety score of 40.8 (SD=12.1; 20=low anxiety to 80=high anxiety) and a mean PPC score of 38.6 (SD=5.1). One month after receiving results, parents whose infants received a definitive diagnosis of GJB2/GJB6 related hearing loss were more likely to agree that they understood why their baby had a genetic test, what the test meant for the future and why initial hearing tests suggested that the infant had hearing loss. Parents whose infants received a definitive diagnosis had higher average PPC scores (41.4; SD=3.4; n=7) versus parents who received non-diagnostic test results (38.3; SD=7.6; n=8). Mean anxiety scores did not differ between the groups (38.0 versus 37.6 respectively). These preliminary data suggest that parents who receive a definitive diagnosis of GJB2/GJB6 related hearing loss for their infant may have better perceived personal control. Increasing perceived personal control may improve parents ability to initiate early interventions that can improve outcomes in their children.

Multi-Institutional Survey to Assess the Need for Genetic Counseling in Pediatric Cardiology Settings-Patient Perspective. *D. Gruber¹, R.S. Cooper², J.D. Menasha³, M. Rutkowski⁴, D.T. Hsu¹, J.G. Davis², J.G. Pappas⁴.* 1) Columbia Presbyterian Medical Center, New York, NY; 2) Weill Cornell Medical Center of New York Presbyterian Hospital, New York, NY; 3) Mount Sinai School of Medicine, New York, NY; 4) New York University School of Medicine, New York, NY.

Advancements in human genetics over the last decade have had a tremendous impact on many fields of medicine, including Cardiology, while presenting a substantial challenge for both health care providers and patients. Our earlier study revealed a deficiency in knowledge of genetics and genetic counseling among the Cardiologists we surveyed, and significant differences in their responses to patients psychological concerns. This project sought to evaluate the genetic counseling needs in pediatric cardiology patients and their families. A patient/parent questionnaire (25 questions) was administered to a cross-sectional survey of pediatric patients (N=117) recruited from various pediatric cardiology care settings. The questionnaire for patients affected with congenital heart disease (CHD) or cardiomyopathy was designed to provide the medical community with information on patients' basic perceptions of recurrence risks, the genetics of their condition, and to assess parents and/or patients level of satisfaction with the genetic counseling provided by cardiologists. Additionally, the questionnaire was designed to demonstrate the extent of the patients willingness to share information with other family members, who could also be affected by the condition. The study revealed a deficiency in patients understanding of the genetics of their condition. In addition, patients expressed an interest in having genetic testing and genetic counseling as well as a willingness to share the results of the testing with other family members. In conclusion, the demonstrated willingness of the patients to participate in genetic testing, genetic counseling and to share information with at risk family members emphasizes the need for accurate patient education regarding the genetics of their condition.

Racial Differences in Acceptance of Genetic Counseling Referrals. *S. Hensley-Alford¹, R. Pollack¹, M. Ford², H. Gordon², L. Lamerato¹, J.R. Roberson¹, D.L. VanDyke³.* 1) Josephine Ford Cancer Ctr, Henry Ford Hosp, Detroit, MI; 2) Baylor College, Houston, TX; 3) Mayo Clinic, Rochester, MN.

The purpose of this study is to describe the clinical experience of the Henry Ford Health System (Detroit, MI) genetic counseling clinic with regard to referral uptake following breast cancer diagnosis. Henry Ford Health System (HFHS) is the largest health system in southwestern Michigan and serves approximately 800,000 patients in the region, 35% of whom are African American. The system operates a multi-disciplinary breast cancer clinic (MDBCC) for women with newly diagnosed breast cancer. During the Clinic, family histories are gathered from women and subsequently reviewed by a genetic counselor. Those with a history suspicious for a breast cancer mutation are sent a referral 6 months post-diagnosis to attend genetic counseling. The genetic counselor tracks all referrals made through the MDBCC for acceptance of genetic counseling. From January 1, 1999 through June 1, 2004, referrals were sent to 233 (61%) Caucasian women, 141 (37%) African American women, and 11 (2%) women of other races. Of those sent a referral, 33 (14%) Caucasian women, 8 (6%) African American women, and 2 (18%) women of other races responded by accepting a genetics consultation. Using the chi-square test, we calculated the associated odds ratio for referral response and the corresponding 95% confidence interval. We found that Caucasians were 2.74 times more likely to respond to referral than African Americans, with a corresponding 95% confidence interval of 1.97-3.81. African American women diagnosed with breast cancer appear to be less likely to attend genetic counseling than Caucasian women diagnosed with breast cancer. Further research is needed to identify the barriers to genetic counseling for African American women.

Asian groups differ in response patterns about relevant health history and perceived causes of disease. J.L.

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The group "Asian" is defined by the U.S. Office of Management and Budget as the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. However, "Asian" is a pan-ethnic identity that is not recognized in Asian countries. The term emerged in the U.S. and blurs national origin distinctions. We questioned whether the broad term "Asian" may be masking differences and hypothesized that groups collapsed within this category differ on health history responses and perceived causes of disease. We tested for differences among Filipino, Japanese, and Chinese adults screened for DNA and blood iron marker abnormalities at the Kaiser Permanente-Hawaii site of the Hemochromatosis and Iron Overload Screening Study (n=594).

Chi-square tests compared the groups on: 1) assigning importance to factors for why "people get sick" (i.e., heredity, the environment, fate or chance, psychological factors, and lifestyle), and 2) answering whether they or their blood relatives have hemochromatosis or iron overload or have been treated by having their blood drawn on a regular basis. In all instances Filipinos were more likely to say they were "not sure" vs. assigning a level of importance or giving a concrete answer, respectively (p.05). Logistic regression controlling for age and gender confirmed that Filipinos differ from Japanese in being "not sure" in all instances (p.05) and from Chinese in all instances (p .05) except "heredity".

In summary, groups that are subsumed in the category "Asian" differ in responses on health history and perceived causes of disease. This may reflect different levels of awareness, or a distinctive response style for Filipinos. These results suggest that genetic counselors working with Asian populations tailor their approach to the specific community being served.

Comparison of two Decision Aids for Hereditary Breast & Ovarian Cancer (HBOC): Knowledge and Decision Conflict in Women and their Family Physicians. *R. Hughes¹, K. McLachlan¹, K. Blaikie¹, J. Liu¹, M. Angen², G. Fick³, A. O'Connor⁴.* 1) Oncology, Univ Calgary, AB; 2) Psycho-social Oncology, Calgary, AB; 3) Community Health Sci., Univ. of Calgary, AB; 4) Epidemiology & Community Health, Univ. Ottawa, ON.

We compared two HBOC decision aids: Canadian Cancer Society (CCS) and Alberta Cancer Genetics Program (ACGP). **SUBJECTS:** New patients (females) referred for HBOC counselling. Referring GPs were eligible if they had no previous contact with the clinic. **METHODS:** Physician/ patient pairs received either the CCS or ACGP decision aids based on GPs gender and year of graduation from medical school. Pre-intervention questionnaires completed and returned prior to receiving the intervention and post-questionnaire. **RESULTS:** 50 GPs invited: 42% declined, 14% no response, 48% agreed. 24 patients invited: 31.3% declined, 14.6% no response, 54.2% agreed. Mean age of participating GPs 49 yr + 13 yr SD. Mean age of patients 42 yr + 14 yr SD; majority married with more than high school education. Patient scores on pre-intervention knowledge questions 35% + 19% SD (CCS) and 30% + 15% SD (ACGP) (t-test $p=0.61$). Post-intervention knowledge scores 50% + 23% SD (CCS) and 90% + 15% SD (ACGP) ($p=0.008$). Increase in knowledge score using paired t-tests ACGP group ($p=0.002$) CCS group ($p=0.29$). GP scores on pre-intervention knowledge questions were 30.6% + 16.7% SD (CCS) and 35.2% + 10.9% SD (ACGP) ($p=0.61$), and post-intervention were 55.6% + 11.1% SD (CCS) and 57.8% + 16.5% SD (ACGP) ($p=0.84$). Increase in knowledge scores not significant using paired t-tests (ACGP $p=0.19$; CCS $p=0.07$). Patient mean scores on Decision Conflict Scale (DCS) were low (<2.5) both before and after the interventions. Factors influencing cancer surveillance choices included test discomfort and opinions of family, friends, doctors and genetic staff. Factors influencing choice for genetic testing included health concerns, family relationships, insurance, test reliability, opinions of doctors and genetics staff. **CONCLUSION:** ACGP decision aid (but not CCS) associated with significant improvement in HBOC related knowledge for patients but not their GPs. Funded by: Calgary Health Region.

Psychosocial Adjustment to Visual Loss in Patients with Retinitis Pigmentosa. A. Levin^{1,2}, D. Jangra¹, R. Thackray^{1,3}, L. Austin¹, A. Ulster^{1,3}, J. Sutherland¹, E. Heon^{1,2}, A. Ganesh⁴. 1) Dept Ophthalmology, M158, Hosp Sick Children, Toronto, ON, Canada; 2) Dept Genetics, Hosp Sick Children, Toronto, ON, Canada; 3) Dept Social Work, Hosp Sick Children, Toronto, ON, Canada; 4) Dept Ophthalmology, Sultan Qaboos University Hospital, Oman.

Objective: To investigate level of psychosocial adjustment to visual loss in patients with retinitis pigmentosa (RP). **Design:** Cross-sectional study. **Methods:** Thirty-three legally blind RP patients participated in the study. Information regarding the patients adjustment to their visual loss was obtained by employing the Psychological Adjustment to Illness Scale (PAIS-SR). Seven psychosocial domains were tested: Health Care Orientation, Vocational Environment, Domestic Environment, Sexual Relationships, Extended Family Relationships, Social Environment, and Psychological Distress. These scores were compared with the psychosocial adjustment of diabetic patients to their illness (normative mean). **Results:** Significantly elevated scores (a high score reflecting poor adjustment) were seen in 4 out of 7 domains and the total PAIS-SR T-Score, compared to the normative mean. The highest relative score was seen in the health care orientation domain (6514, p0.001) followed by vocational function (6111, p0.001), social environment (589, p0.001) and extended family relationships (559, p0.05), respectively. The total PAIS score was significantly elevated (588; p0.001) compared to the normative mean. **Discussion:** Patients with RP have difficulties in adjusting to their visual loss particularly with respect to health care orientation, vocational environment, social environment, and extended family relationships. They face more difficulties than diabetic patients in adjusting to their illness. This study identifies problems specific to patients with RP suffering from visual loss.

Parental Attitudes Toward Genetic Testing In Their Infants With Hearing Loss. *A. Martínez¹, M. Fox¹, B. Crandall¹, N. Shapiro¹, M. Telatar¹, Y. Sininger¹, W.W. Grody¹, L.A. Schimmenti², C.G.S. Palmer¹.* 1) University of California Los Angeles, Los Angeles, CA; 2) University of Minnesota, Minneapolis, MN.

We present preliminary data on parental knowledge, understanding of outcomes of genetic testing, and attitudes toward genetic testing for hearing loss as part of a study assessing the timing and impact of providing Connexin26/30 testing and counseling in the Early Hearing Detection and Intervention (EHDI) process. Families with a child under 3y with potential or diagnosed hearing loss and no other conditions are enrolled into the prediagnosis (failed newborn hearing screening, prior to diagnosis) or the postdiagnosis (diagnosed hearing loss) group. Genetic testing is considered in the context of genetic counseling. Results are disclosed in a second genetic counseling session. Parental data are collected after enrollment, 1 month and 6 months post result disclosure. Data on 50 parents (33 families) in the postdiagnosis group are described. Following enrollment the majority of parents understood heterogeneity and inheritance concepts and felt there were benefits of genetic testing. Some parents were concerned about potential harms of testing. 9 babies had biallelic Connexin26 mutations: 1m after disclosure 90% of parents of these babies indicated their child definitely had Connexin26-related hearing loss, and 100% indicated that genetic testing helped them understand the cause of hearing loss, and that the recurrence chance is 25%. Hearing loss of 24 babies was not explained by genetic testing: 1m after disclosure, ~80% of those parents indicated that their child did not have Connexin26-related hearing loss, and 70% indicated that the cause of their baby's hearing loss was undetermined, and that the test did not help them understand the cause of hearing loss. There was considerable variability in their understanding of recurrence chance. Conclusions: Incorporating genetic testing into the EHDI process will be welcomed by some parents. However, implementation should be carefully considered as these results illustrate that testing raises some concerns and that negative results are difficult to understand. Strategies for improving communication of negative results are needed.

Parental Perspectives on the Causes of Autism Spectrum Disorder. *L. Mercer*^{1,3}, *S. Creighton*^{1,3}, *J.J.A. Holden*^{2,3}, *M.E.S. Lewis*^{1,3}. 1) University of British Columbia, Vancouver, BC, Canada; 2) Queen's University, Kingston, ON, Canada; 3) Autism Spectrum Disorder-Canadian American Research Consortium (ASD-CARC; www.autismresearch.ca).

Autistic Spectrum Disorders (ASDs) are complex neurobehavioral disorders with many biologic causes, including genetic, syndromic and environmental contributors. Such etiologic heterogeneity impacts considerably upon a parent's need to understand and have an explanation for their child's diagnosis. A cross-sectional semi-quantitative survey was designed to investigate parental views on the cause of ASD in their child. Such research has not been conducted in almost 10 years and, therefore, recent data is warranted for effective patient support and counseling in the clinical setting. The study surveyed parents with at least one child with ASD. Questions in the survey consisted of various format types such as fill in the blank, dichotomous, Likert response, cumulative/guttman and nominal. Parents were encouraged to include comments. Among the 41 parents who filled out the questionnaire, genetic influences (90.2%), peripartum factors (68.3%), diet (51.2%), prenatal factors (43.9%) and vaccines (43.2%) were considered most contributory to ASD causation. Underlying genetic susceptibilities and triggers by a myriad of environmental factors (e.g. chemicals, food additives, illness during infancy and psychological factors) were frequent assertions by parents. Parents attribute many factors to, and often have strong beliefs about, the cause of their child's ASD. Parents reported inaccurately high recurrence risks, misperceptions of the contribution of various factors, feelings of guilt and blame regarding their child's diagnosis, as well as a lack of advocacy for genetic counseling by non-geneticist professionals. Genetic counseling would certainly benefit ASD families through the provision of information regarding diagnosis, the role of genetics and recurrence risks. This study offers clinicians and researchers further insight into what parents believe contributed to their child's diagnosis of ASD. Supported by research grants from CIHR.

The Use of Preventive Measures Among Healthy Women who Carry a BRCA1 or BRCA2 Mutation. K.A.

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Introduction: Tamoxifen has been shown to significantly reduce the risk of developing breast cancer in both high-risk women and those with a BRCA1 or BRCA2 mutation. The purpose of this study was to examine uptake rates of tamoxifen in BRCA1 and BRCA2 mutation carriers, to assess concerns about taking the chemopreventive drug, and to assess knowledge about tamoxifen. **Methods:** Women with a known BRCA1 or BRCA2 mutation, with no prior diagnosis of breast or ovarian cancer, were mailed a study specific questionnaire. The questions included in the questionnaire were based on input from health care providers and women seen in the genetics clinics. The items assessed the women's knowledge of tamoxifen, their usage of tamoxifen, and their need for additional information on tamoxifen. **Results:** Eighty-one of 125 eligible women returned the questionnaire (64.8%). Ten women (12.3%) had used tamoxifen and eight women had used raloxifene (9.9%). Twenty-two women (27.2%) had undergone prophylactic mastectomy and 54 women (66.7%) had had an oophorectomy. Twelve women (14.8%) did not recall discussing tamoxifen. Thirty-five women (58.3%) said their doctors had not provided them with sufficient information about tamoxifen to consider its use. Fear of side-effects was the most common reason for not taking tamoxifen. **Discussion:** Although tamoxifen has been shown to be effective in preventing breast cancer in women with a BRCA1/2 mutation, the majority of women choose not to take the drug. Fear of side-effects and a lack of education regarding tamoxifen were reported as the reasons for not taking the drug. Women were much more likely to elect preventive surgery than to take a preventive drug. Health care providers need to be aware of the misconceptions associated with tamoxifen, and provide accurate information about the risks and benefits so that women can make informed decisions about chemoprevention.

Patient knowledge, risk perception, health behavior and interest in genetic counseling and testing for familial melanoma. *K.B. Niendorf¹, B. Somoano⁴, W. Pirl², A. Muzikansky³, A. Sober⁴, H. Tsao^{1,4}.* 1) Center for Cancer Risk Analysis, Massachusetts General Hospital, Boston, MA; 2) Department of Psychiatry, Massachusetts General Hospital, Boston, MA; 3) Biostatistics Center, Massachusetts General Hospital, Boston, MA; 4) Department of Dermatology, Massachusetts General Hospital, Boston, MA.

Mutations in the CDKN2A gene have been identified in 20-40% of melanoma-prone families. Although clinical testing for CDKN2A mutations is available on a commercial basis, published recommendations propose that this genetic testing largely remain as part of research protocols. Provision of these test results, even on a research basis, is aided by an understanding of the patients' baseline genetics knowledge, risk perception, health behavior and interest in genetic testing and counseling for familial melanoma. For this reason, we queried patients with either family histories of melanoma or personal histories of multiple melanomas prior to provision of genetic counseling and testing for CDKN2A mutations. To date, 205 potential participants have been identified and 67 have completed the questionnaire. Prior to receiving genetic counseling as part of the study, most participants reported hearing/reading only a little bit or nothing regarding melanoma susceptibility genes. Sixty-eight percent of participants qualitatively rated their risk as high or very high for having a mutation associated with increased melanoma risk. Nearly all of the participants had at least one clinical skin examination performed in the past year; however, 61% reported being a little or moderately tanned at the end of summer. The majority of participants wished to pursue familial melanoma genetic counseling (64%) and genetic testing (66%). Thus, many individuals with familial melanoma risk indicate a lack of knowledge of melanoma genetics yet feel that they are at high-risk of carrying a hereditary melanoma predisposition genetic mutation. These data suggest that at-risk patients have a need for familial melanoma genetics information while also offering a foundation for understanding the potential utility of genetic counseling and testing.

Evaluating a family cancer registry: Benefits, distress and risk. *S. Petzel¹, A. Leininger², D. Rothenberger², L. Carson¹.* 1) OB-GYN, University of Minnesota, Minneapolis, MN; 2) Minnesota Colorectal Cancer Initiative, Minneapolis, MN.

PURPOSE. Psychoeducational outcomes were evaluated for a Familial Colorectal Cancer (CRC) Registry. CRC patients/family provide family history and receive written CRC and genetic risk- and surveillance-information.

METHODS. A survey mailed to enrollees measured: 1) Registry usefulness, based on Social Learning Theory (Likert scale/1, low-to-5, hi); 2) Lifetime (subjective) CRC risk, estimated by category (Ave, Incr, Hi/word) and linear analogue scale (0-100%/numeric); 3) Emotional distress (Impact of Event Scale and a Likert scale/0, no-to-10, hi). Risk-seriousness and health-demographic information were collected.

RESULTS. Subjects: 41 males and 74 females, primarily increased CRC-risk, Caucasian, ages 25-83 and in 4 health groups: CRC, CRC precursors; non-CRC; no cancer. Most (93%) rated registry pros/cons. Overall usefulness was not correlated with perceived risk, risk-seriousness, distress or demographic-health variables; pros were not correlated with cons. Notable pros: promoting coping and understanding cancer risk; probable barriers: negative attitudes. Cons were associated negatively with education, marital status, income; positively with distress (p.05). Distress was low and not associated with risk-recall accuracy or health-demographic variables except women were more distressed (p.0001). Risk was over-reported; accuracy, low. Word estimation was more accurate than numeric (41% vs 14%; p.003). Risk seriousness (p.0001) and subjective risk (p.02/word, p.0004/%) were associated with distress: the more serious the perceived risk or the higher the risk estimate, the greater the distress.

CONCLUSIONS. Some goals are being met: providing useful information without promoting distress is successful; promoting accurate risk perception less so. Low distress suggests this is not an emotionally vulnerable population or those electing to receive risk information were relatively resilient. In diverse at-risk cancer populations effective genetic education and counseling may require individualized tailoring.

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Genetic and reproductive counselling for multiple sclerosis:current model and pregnancy register. *A.D. Sadovnick.* Prof/Med Genet/Div Neurology, Univ British Columbia, Vancouver, BC, Canada.

While the etiology of multiple sclerosis (MS)remains unknown, there is increasing evidence for a genetic component to both susceptibility and clinical outcome. Confounding factors include overall (not interfamilial) environment, gender of transmitting parent, family structure, and age of onset. In addition, disease modifying therapies (DMTs)are increasingly being recommended to individuals in the very early stages of disease. As MS shows a clear female predilection and strikes during the prime reproductive years, genetic recurrence risk information and reproductive counselling are increasingly being requested. A protocol for such counselling will be presented and will incorporate the most recent information about factors influencing recurrence and the safety of DMTs at conception, during gestation, and when breastfeeding. In addition, the need and paradigm for a disease-specific pregnancy register will be presented.

Prenatal Testing for BRCA1 Mutation. N.Scanlan¹, J.H.Jung¹, P.J. Ainsworth¹, G.Sheridan¹ 1) Cancer Genetics, London Health Sciences Center, London, ON, Canada. *N. Scanlan, J.H. Jung, P.J. Ainsworth, G. Sheridan.* Cancer Genetics, London Health Sciences Center, London, ON, Canada.

Prenatal testing for a familial BRCA1 mutation to determine the risk of an inherited predisposition to breast and/or ovarian cancer in the fetus represents a controversial ethical issue, with a view to selective termination of pregnancy in case of a female carrier. A 34-year-old G1P0 woman was referred for prenatal molecular testing for BRCA1. Two years previously, the woman and her partner had been counselled regarding the identification of a deleterious familial BRCA1 mutation (2561_2562insGC) carried by her partner. At that time the couple questioned the availability of prenatal testing if a pregnancy was achieved. They indicated that they wished to proceed with prenatal testing because they would consider the termination of a female mutation carrier. The couple was seen for additional genetic counselling to discuss medical, genetic and ethical issues related to prenatal BRCA testing and to discuss their reasons for wanting testing in this instance. The couple was referred to an obstetrician, who was aware of the family history, for further discussion of issues related to prenatal screening. The couple achieved a pregnancy and at 11 weeks gestation were seen for counselling related to age risk, chorionic villi sampling, amniocentesis procedures, BRCA inheritance risks and molecular testing options. Both prospective parents requested identification of the sex of the fetus as well as the BRCA1 mutation status. An amniocentesis was performed at 16 weeks gestation and the results confirmed that the mother was bearing a male fetus that was identified as a BRCA1 carrier. This information was provided to the couple who opted to proceed with the pregnancy. The complex medical genetic and ethical issues raised in this case illustrate the importance of close inter-disciplinary collaboration required in prenatal testing for adult onset diseases.

GENETICS MADE EASY Non-profit divulgation web on human genetics. *M.T. Sole - Pujol^{1, 2}, J.M. Carrera², J.M. Cantu³, F. Sole⁴, J. Antich¹.* 1) Centro Genetica Medica, Barcelona (B), Spain (S); 2) Servicio de Medicina Fetal. Instituto Universitario Dexeus, (B), (S); 3) Division Genetica. Instituto Mexicano del Seguro Social. Guadalajara. Mexico; 4) Laboratorio de Citogenetica y Biologia Molecular. Hospital del Mar, (B), (S).

Genetics Made Easy <http://www.lagenetica.info>, is a non-profit informative web on human genetics that has been written with the aim of bringing the scientific community closer to the general population. The web is an excellent complement to genetic counseling and a very useful teaching tool to clinicians and other healthcare professionals, in order to complement medical personal consultations as you will see through the index web, regardless of their area of expertise, as genetically inherited disorders are known across all medical specialties. Supplemented by static pictures and flash animation, at this moment is currently available on the Net in both Spanish and English, and during this year in Chinese. The web contains the information that any couple may need to know before embarking in parenthood, or if they have already children with some type of malformation or hereditary disorder. Additionally, a need arises for the general population of any age to be well informed, starting ideally at school age. The index web is: * Introduction, * The origin of life, * Cell specialization, * Chromosomes, * How do we acquire our inheritance, * What is heredity, * Types of inheritance, * Why do disorders develop, * What happens when our recipes combine with our partners recipes?: here on page 3 through the tree you can explain the specific mendelian risk for a family, complemented by flash animation, and links to look for the specific disease. * And how can we use this vast knowledge and benefit from it: On page 2 you have a tree with all the assisted reproduction techniques that we use at this moment, and in page 3 and 4 some of the risks that they conduct. * Origin of hereditary disorders: Go inside the yellow bar chromosomal disorders. * Prenatal diagnosis techniques: Complemented by flash animation, * Cloning, * Questions, * Links of interest: To be sure people get information from serious places, * Further reading, * Foreword. FORUM.

Pediatricians' knowledge and views on genetic counseling for cancer predisposition. *L. Squirrell^{1, 3}, A. Kaiser^{1, 3}, H. Druker^{1, 3}, A. Novokmet^{1, 3}, S. Meyn^{2, 3}, R. Weksberg^{2, 3}, D. Malkin^{1, 3}.* 1) Haematology/Oncology, The Hospital for Sick Children, Toronto, ON, Canada; 2) Clinical Metabolics and Genetics, The Hospital for Sick Children; 3) Department of Pediatrics, University of Toronto, ON, Canada.

Genetic factors may play a role in a significant proportion of childhood cancers, and genetic counseling for cancer predisposition can accelerate medical intervention and allow advance planning. The pediatrician must often identify a child at risk for cancer, and effectively counsel and refer for genetic testing. We aimed to assess the knowledge, views, current practice and referral behaviours that pediatricians hold of genetic testing for cancer predisposition, and to evaluate the effectiveness of a cancer genetics lecture given at a Genetics CME 3-day update as an intervention tool. A questionnaire was distributed to pediatricians before and after they attended the lecture, and to an expert control group of pediatric oncologists, geneticists and fellows. 34 pediatricians responded to the pre-lecture questionnaire, and 31 to the post-lecture questionnaire (response rates = 60.7%, 55.4%). At this point 19 experts have responded to the questionnaire (response rate = 21.8%). On a knowledge-based test of cancer genetics, pediatricians scored 65.9% [95% confidence interval (CI), 61.1-70.7] before attending the lecture, and 76.0% (95% CI, 70.5-81.5) afterwards. The expert control group scored 76.8% (95% CI, 67.1-86.5). 96.8% of pediatricians felt that it is very important or important to detect genetic disorders in their patients. 100% of respondents did not feel they had sufficient knowledge of genetics to provide accurate counseling on cancer predisposition, yet 51.6% of pediatricians had independently provided genetic counseling, and 90.3% of pediatricians had referred a patient for genetic counseling. The results suggest that pediatricians recognize the importance of genetic counseling for cancer predisposition but may not hold the knowledge to effectively identify, counsel and refer their patients. Educational training is important to ensure cancer genetics services are accessed appropriately.

Counseling in cancer genetics using teleconferencing technology a single center experience of 130 consultations.

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The demand for cancer genetics services has increased dramatically over the last decade. While tertiary referral centers have been set up in the UK, patients are often long distances from these. Moreover there are few specialists in cancer genetics and time spent traveling to peripheral clinics reduces their work efficiency. We sought to reduce patient traveling time and increase the specialist efficiency by developing remote consultation clinics using real-time videoconferencing technology. The first clinic was set up in August 2002 and rapid expansion has led to the creation of three further telemedicine cancer genetic clinics covering the region. We now have two years experience in using telemedicine for cancer genetics and to date 130 consultations have taken place, 66% breast or breast/ovarian cancer families, 31% colorectal cancer families. Ten consultations were for predictive tests including two in children at 50% risk of Familial Adenomatous Polyposis. A questionnaire-based comparative study with face-to-face consultations showed no significant differences in levels of comprehension, anxiety levels and overall satisfaction with the consultation. We conclude that telemedicine is an effective and efficient tool in cancer genetics which can be used to deliver care over long distances and has the potential to give remote communities direct access to specialist services. We are currently looking to develop international collaborations to establish clinics outside the UK.

The Utility of NF 1 Mutational Analysis in the Clinical Setting. *E.H. Zackai¹, D.M. McDonald-McGinn¹, K.L. Ciprero¹, L. Medne¹, M.K. Maisenbacher¹, B. Korf², L. Messiaen².* 1) Clinical Genetics Center, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Medical Genomics Laboratory, University of Alabama at Birmingham, AL.

We reviewed the utility of NF1 mutational analysis, having a 95% sensitivity in NF1 patients fulfilling the NIH diagnostic criteria during the first 10-month period following its clinical availability in the US. 107 patients clinically evaluated using the NIH consensus criteria were divided into 4 categories: A-clinically unaffected, but presenting with atypical cafe-au-lait (CAL) spots (50); B-segmental NF (4), isolated pseudoarthrosis (2) or isolated plexiform neurofibroma (1); C-clinically affected, fulfilling NIH criteria (17); and D- not meeting criteria requiring follow-up (33). 23/107 had mutational analysis completed. In Category A, 3 patients tested to allay parental anxiety were negative. In Category B, 3 patients (2 likely having segmental NF and 1 with plexiform neurofibroma) were negative. In Category C, 5 patients underwent mutational analysis and a mutation was identified in all of them. In Category D, 12 patients were tested. In 5 children under age 1 year with CALs who were highly suspect, but were too young to have developed additional clinical signs, an NF1 mutation was found. One 7 yo with 3 CALs and axillary freckling had a mutation. In a sporadic 15 yo with CALs and axillary freckling a novel conserved missense mutation was found. Four patients with only CALs were negative. A 33 yo woman with >6 CAL spots was negative for the mutation identified previously in her sister fulfilling the NIH criteria. We believe that mutational analysis is a useful tool when combined with the clinical exam. It can: allay parental anxiety in patients with normal exams and in cases presenting with isolated features such as CAL-spots only, a plexiform neurofibroma or pseudoarthrosis; confirm a clinical diagnosis; and assist in the evaluation of children who are followed over time whose exam is not clear. Furthermore, mutation analysis allows for early diagnosis, eliminating long periods of uncertainty. Lastly, it allows one to provide definitive genetic counseling in clinically diagnosed cases.

Lessons learned: relevance of data warehousing to clinical research. *M. Szybowska, S. Hewson, D. Wherrett, T. Pyle, R. Babul-Hirji.* The Hospital for Sick Children, Toronto, Ontario, Canada.

In a healthcare setting, a data warehouse (DW) is an organized electronic database housing data which may be accessible to clinicians, administrators, information services, and clinical researchers. The DW at our centre was established in 1995 permitting ongoing collection and segregation of data since Oct/96. Today, the DW stores full demographic data for all patients that visited the hospital. Of direct relevance to clinical research, the DW also stores details of patient visits and diagnoses, admission and discharge dates, lab results, procedures and information on attending physicians. The standardized data is updated daily, time-stamped providing the means for historical analyses, and managed so that confidentiality is maintained. Non-confidential reports are available to all hospital staff. Reports necessitating access to confidential information require additional authorization. For ease of manipulation, all reports can be downloaded directly into available software. As part of our approved clinical research, we approached medical records to identify our patient population. As the data maintained by the hospital medical records pertains only to in-patients, we turned to the DW for further assistance. The electronic nature of the DW search allowed us to quickly identify our patient population and review only specific charts to confirm the diagnosis. The DW was able to provide a greater number of patients that met our inclusion criteria, thereby increasing our sample size. This was accomplished by searching lab test data specific to the diagnosis that we were studying and proved to be very effective. Clinical research is key to enhancing health and patient care. The evolution of the DW into the storage headquarters for data is the powerful tool that tertiary institutions performing clinical research have been awaiting. The use of the DW in clinical research may well become the norm in the future. From our experience, the DW dramatically cut down the time necessary to perform a patient population search while providing thorough, inclusive, and up-to-date data. In the long run, data warehouses are of direct benefit to clinical research and healthcare.

Genetic education for individuals at risk for Alzheimer's disease: a pilot study. *L. Cortellini¹, T. Moscarillo¹, R. Go², H. Holt², J. Stoler³, D. Blacker¹*. 1) Psychiatry, Mass General Hosp, Charlestown, MA; 2) Epidemiology, U Alabama, Birmingham, AL; 3) Genetics and Teratology, Mass General Hosp, Boston, MA.

For those at risk for Alzheimer's disease (AD), increasingly complex genetic information is a major concern. To address this concern, we developed educational materials for at-risk individuals and piloted them in a sample of 276 unaffected relatives recruited from a genetic linkage study (mean age 61, sd 9, range 40-81; 26% male; 97% white, 3% black; mean education 15 yrs, sd 3.5). Subjects were assessed at baseline, immediately after a randomized educational intervention (AD genetic educational video + pamphlet vs. the pamphlet alone vs. a placebo pamphlet), and 3-6 months later. We examined self-assessment of AD genetic knowledge, and also constructed three knowledge scores regarding 1) overall genetics, 2) AD genetics, and 3) AD general information. We also constructed two genetic testing attitude scores indicating 1) interest and 2) worry. At baseline, a substantial number (~40%) in all three groups felt they knew nothing at all about AD genetics and few (~10%) felt they understood it fairly well. At follow-up, the placebo group changed little, but both intervention groups, particularly video, improved considerably. With regard to objective knowledge, baseline scores in all knowledge domains were similar across the three groups with a fair to good understanding of general genetics (mean score ~6/9), a poor understanding of AD genetics (~1.5/7), and a fair level of general AD knowledge (~4.5/9). At first follow up, there were modest increases in knowledge of general genetics and general AD in all three groups, but a marked increase (~58%) in AD genetic knowledge among those in the two intervention groups. With regard to attitudes, there was little change in worry in any group, but a marked decrease in interest in both intervention groups, particularly video. Future results will include statistical analysis of these preliminary findings and analysis of the 3-6 month follow-up data. Effective educational methods, like this one appears to be, will be critical to any future treatment strategies entailing genetic testing for patient selection or treatment optimization.

A Pilot Study to Evaluate an Internet-based System of Patient Education and Informed Consent for Genetic Studies. *F. Facio*¹, *P. Young*², *R. Baum*¹, *C. Palmer*¹, *N. Kauff*¹, *K. Hurley*¹, *H. Peter*¹, *A. Schluger*¹, *P. Agre*¹, *N. Ellis*¹, *M. Robson*¹, *M. Paisner*², *P. Manasco*², *D. Wang*², *K. Offit*¹. 1) Clinical Genetics Service, Memorial Sloan-Kettering Cancer Center, New York, NY; 2) First Genetic Trust, Deerfield, IL.

As genomic medicine expands, novel strategies to supplement genetic counseling may be needed. We assessed the feasibility of using a secure Internet-based process of patient education and informed consent for genetic studies. Individuals of Ashkenazi Jewish descent previously counseled in our clinic for *BRCA1/2* learned about colon cancer and the *APC11307K* allele (associated with an increased risk of colon cancer) via an Internet Education Tutorial (IET), including an interactive knowledge questionnaire. After completing the IET, participants accepted or declined testing for *APC11307K* via an electronic informed consent (EIC), and were asked to complete an electronic satisfaction questionnaire (ESQ). Internet services were provided by First Genetic Trust (FGT). 34 individuals enrolled between 2/03-2/04. Participants demonstrated a high level of information retention with 33/34 answering at least 7/9 questions correctly in the knowledge questionnaire. 33/34 accepted testing for the *APC11307K* using the EIC. 14/33 eligible participants completed the ESQ. Of these, 50% preferred receiving genetic education via the Internet than in person. 86% felt the IET was easy to understand and contained all the information they needed to decide about *APC* testing. 86% liked very much/felt OK using the EIC. 57% felt extremely/very confident that their genetic testing information would be confidential when using the FGT system. Overall, 21% reported being extremely satisfied, 57% very satisfied and 21% somewhat/not at all satisfied with this Internet-based system. Psychometric measures are in progress. These findings demonstrate that it is feasible to provide patient education and informed consent for low penetrance genetic markers using an Internet-based system. Future studies are needed to establish the best combination of in-person counseling and computer-based education to meet the informational demands of the genomic era.

A Web-Based Educational Learning Module for Fabry Disease. *J. Feiger¹, C. Barash², M. Taylor¹*. 1) Adult Medical Genetics Program, University of Colorado, Denver, CO; 2) Genetics, Ethics and Policy Consulting.

Background: Fabry disease (FD) is a rare X-linked recessive inherited metabolic condition unfamiliar to many general physicians. As there is now enzyme replacement therapy for this serious condition, there is a need to improve physician familiarity with and understanding of FD. We developed and tested a web-based educational learning module designed to target primary care physicians; the site also contains information intended for patients, families, and consumers with a moderate level of medical literacy. **Methods:** Our goal was to develop an effective yet easy to use, educational introduction to FD. The website contained 5 major topics: overview, signs and symptoms, diagnosis, genetics, and management. Video interviews of FD patients were also included to illustrate barriers to early detection. Educational content was designed to increase indices of suspicion, stress variability of clinical presentation, introduce state of the art disease diagnosis treatment, and general management issues, and improve understanding of the genetic basis of FD. To determine learning effectiveness pre and post-tests were used to measure baseline and knowledge increases. Our study population consisted of 51 internal medicine residents (IMR) and medical students (MS) from the University of Colorado. The content, delivery and effectiveness measures satisfied CME requirements and future users can obtain free CME credit. **Results:** Users spent 30 minutes on average reviewing the content; a 21% improvement in knowledge occurred ($p > 0.001$) across all categories of questions (assessed by pre and post test score differences); males and females showed equal improvements in knowledge scores; IMRs showed greater score improvements than the MSs ($p = 0.002$). Participant feedback was positive and the web-based medium for education was well received by participants. **Conclusions:** Our web-based approach to educating IMRs and MSs about a rare genetic condition resulted in general improvements in short-term knowledge and understanding of FD. Data demonstrate that this tool is effective and satisfying, suggesting that this model could be adapted to other diseases.

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Development of an educational platform in genomic medicine in Mexico. *S. March*¹, *I. Silva-Zolezzi*¹, *U. Lopez*¹, *A. Lopez*¹, *L. Abreu*², *G. Jimenez-Sanchez*^{1,2,3}. 1) National Institute of Genomic Medicine, Mexico; 2) National Autonomous University of Mexico (UNAM), School of Medicine, Mexico; 3) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD.

Mexico develops a national platform in genomic medicine based on the genomic structure of the Mexican population and their medical needs, headed by the National Institute of Genomic Medicine. It includes educational programs for professionals and the general public. The former includes a joint program with the National Autonomous University of Mexico that has led to the first three graduate courses in genomic medicine in Latin America: Introduction to genomic medicine, Genomic applications in pediatrics and Genomic applications in internal medicine (<http://www.facmed.unam.mx/programas/index.html>). These courses review medical implications of genomic medicine in different areas of medicine. Our students have different educational backgrounds, including computational sciences, engineering, nutrition, pediatrics, oncology, chemistry, public health, neurology and, cardiology. Through these courses, students develop research proposals applying genomic medicine to a national health problem. Our program includes over 20 faculty members. These efforts will lead to the first doctoral program in genomic medicine in Latin America in the spring of 2005. Materials from our graduate courses, over 15 publications, 100 video-lectures and other tools for academic use have been incorporated to our web portal (www.inmegen.org.mx). This electronic resource has turned into a major educational tool in genomic medicine for Latin America. In the last 10 months, there has been total of 683,000 consultations with an average of 107 sessions per day. In addition, 37,083 publications and videos have been downloaded from this portal. Over 80% of the users are from Latin America. To ensure participation in this educational platform of faculty and students from across the country, in June 2003 the Mexican Society of Genomic Medicine (SOMEGEN) was founded (www.somegen.org.mx). This educational effort is envisioned as a strategic instrument to support the national platform in genomic medicine developing in Mexico.

Program Nr: 1436 from the 2004 ASHG Annual Meeting

Communication workshops for geneticists: facilitating public engagement. *K. Mathieson, D. Donnai, H.R. Middleton-Price.* Nowgen, St Mary's Hospital, Manchester, United Kingdom.

The medical applications of human genetic research are often in the public eye. At Nowgen (the North West Genetics Knowledge Park) we are committed to engaging in dialogue with the public about these advances; the myths and the reality. In 2002 a survey of BSHG (British Society of Human Genetics) members indicated that a majority would like to spend more time communicating their work with the non-specialist public and many requested further training to help them. In order to encourage members of the BSHG and other geneticists to talk to community groups and schools about their work, Nowgen is running a programme of communication workshops. We ran our first workshop in February and geneticists from a variety of disciplines attended. The workshop included interactive sessions on: becoming more aware of the needs of audiences, making presentations more creative, avoiding using loaded language, finding alternatives to scientific jargon and on facilitating debate. It received positive evaluation and future workshops will be developed based on the findings of this first experience. For all the workshop participants who want to practise their new found skills, Nowgen liaises with a school or community group to arrange a visit.

Program Nr: 1437 from the 2004 ASHG Annual Meeting

Essentials of Clinical Cancer Genetics: Internet-Based Curriculum. A. Matthews, T. Nosek, K. Papp, N. Wolf, G. Wiesner. Dept of Genetics, Case Western Reserve Univ, Cleveland, OH.

Cancer genetics evaluation and testing has changed medical management for at-risk populations. However, the lack of genetics education and experience has left physicians ill equipped to provide up-to-date cancer genetic information to their patients. To improve the education of future physicians, we are developing a dynamic multimedia cancer genetics medical school curriculum, entitled *Essentials of Clinical Cancer Genetics*. This interactive curriculum will be embedded in the Internet-based Case School of Medicine eCurriculum and will provide up-to-date information regarding the principles of cancer genetics and their application to clinical practice, genetic counseling, and patient care. It will also address the ethical, legal, and social issues inherent in counseling for cancer genetics. The text portion of the *Essentials* will be richly supported by illustrations, animations and clinical case studies that will help to illustrate the core objectives as well as more subtle concepts. Video clips and case studies will illustrate complex genetic counseling issues such as concerns and fears about the meaning of a cancer diagnosis, dealing with loss, grief, and discrimination. Students will be tested for competence in cancer genetics factual knowledge, comprehension and problem solving. Students completing the *Essentials* (the Intervention group, N= 140) will be assessed at these three tiers of competency and their performance compared to a group of historical controls, i.e., students who received the current nominal level of cancer genetics instruction without exposure to the *Essentials* curriculum (N=140). Moreover, the interactive nature of the *Essentials* will allow for the use of more innovative testing methodologies. Once completed, the *Essentials* will also be made available to other medical schools or other learners in the future. This work has been supported by NCI R25 CA092357-01A2.

Program Nr: 1438 from the 2004 ASHG Annual Meeting

Making Written Information More Accessible. *S.G. Nicholls¹, H.R. Middleton-Price¹, D. Donnai¹, L.M. Davies^{1, 2}.*
1) The North West Genetics Knowledge Park (Nowgen), St Marys Hospital, Manchester; 2) Health Economics Research at Manchester (HER@M), University of Manchester, Manchester.

The NHS and UK legislation now recognise the importance of involving patients in their own treatment and in NHS policy. Nowgen is committed to an active programme of service user and public engagement. Good communication is critical to fulfilling this objective: a lack of accessible resources reduces our capacity for engaging service users and the public in an informed dialogue. Reviews of information produced by scientists and doctors for the public suggest much of what is written is at a level beyond those to whom the material is targeted limiting its effectiveness and value. The objective of this study is twofold: 1) Review of current resources to develop practical guidelines for producing leaflets that are readable, accessible and acceptable to users of the NHS and the public. 2) To use these guidelines to review a sample of existing leaflets from clinical genetics services in terms of readability and accessibility. These Nowgen guidelines include ways to improve the visual clarity of the document, accessibility of language and document layout and attractiveness. We also highlight alternative options to standard print as a potential way of improving engagement. Initial analysis of information leaflets indicate that they vary in presentation and accessibility. None have met all the guidelines.

Building genomics capacity in the public health workforce in Minnesota: Advice to the Minnesota Department of Health from key people. *K.P. Oehlke¹, M.A. Casey, PhD², R. Krueger, PhD³.* 1) State Genomics Coordinator, Minnesota Dept Health, Minneapolis, MN; 2) Independent Consultant, St. Paul, MN; 3) University of Minnesota, St. Paul, MN.

The Minnesota Department of Health (MDH) is working to build capacity to assure appropriate integration of genomics into public health activities in Minnesota. To better understand what needs to be done related to this, five telephone focus groups were held in May 2004 with 23 people including researchers, educators, public health practitioners, healthcare providers, and healthcare advocates from across Minnesota. The purpose was to solicit input that could be used to shape genomics capacity-building activities. Questions included: 1) What issues are important related to genomics? What are your concerns? 2) What role should MDH play related to genomics? 3) What gathering or meeting format do you find most useful? Issues related to genomics were ranked from one (not at all important) to five (extremely important). The three most important issues were 1) ethical, legal and social issues (ELSI) (4.8/5); 2) public policy issues related to genomics (4.3/5); and 3) genomics in health education, health promotion and disease prevention (4.0/5). Participants advised MDH to provide education and training to audiences within and outside MDH, to provide support to public health practitioners to help them incorporate genomics into their existing programs, to maintain genomics data securely and to have a sound rationale for genomics data collection. Education should focus on basic genomics information, genomics in public health, ELSI, and updates on advances. MDH should support efforts for anticipating ELSI and assuring that public policies balance individual, research and public health priorities. Any meeting on genomics aimed at the public health community should have a defined audience, a clear purpose, a carefully considered venue and something special to attract the desired audience.

Clinical presentation based teaching of the metabolic diseases will speed diagnosis and successful treatment of rare inborn errors of metabolism. *G. Porras, A. Perszyk.* Pediatric Multispecialty Ctr, Univ Florida, Jacksonville, FL.

Metabolic disorders that present acutely in children are rare. Far more common are ingestions, injuries and infections in this age group. Therefore, genetic disorders of metabolism need to be discussed in the differential diagnosis of acutely ill infants and toddlers, to improve early recognition, confirmatory testing, and successful treatment. Purpose: To teach a practical approach to when and where to do metabolic testing. Rapid assessment and initial timing of the acute work-up for a child or infant that presents with altered mental status, hypoglycemia, shock or cardio-respiratory collapse is important to teach in a pediatric residency training program, and to nursing staff at the childrens hospital. Method: Pediatric Emergency Department and Pediatric Intensive Care Unit are supported with 24-hour call coverage utilizing dedicated genetic staff skilled in the metabolic work-up, diagnosis, intervention and treatment. Coordination of core lecture series for the pediatric house staff, critical care fellows, and ICU nursing in-service program. Education of Neonatal ICU attending and nursing staff, as well as the obstetrical attendings and residents all have a role in recognizing a metabolic disorder. Provided are algorithms and initial lab work-up for metabolic disorders for use with a palm pilot device. Conclusion: Awareness of metabolic disorders is the first step in the diagnosis of these conditions. Rapid response to resident calls and attending inquiries has improved the yield of diagnosis, timely interventions, and immediate and long-term outcomes for children that present acutely ill. Residents need access to and support for thinking of genetic and metabolic diseases. Correct diagnosis can yield many benefits to the child, family, future children, and the institution that cares and manages these children. A sensible clinical approach has been the basis for more than 10 years of didactic education. This approach is a significant reason for the correct diagnosis and the long-term treatment and support for children with metabolic disorders.

Barriers to use of cancer family history data by primary care providers. *M. Wood*¹, *A. Stockdale*², *B. Flynn*¹. 1) Hematology/Oncology, University of Vermont, Burlington, VT; 2) Education Development Center, Boston, MA.

Purpose: The cancer family history is key to identifying syndromes and recommending cancer screening. We sought to understand the barriers to obtaining a complete and accurate cancer family history in the primary care setting. **Methods:** Interviews with primary care providers (PCPs) in rural Vermont, urban and suburban Boston were performed by a medical anthropologist (AS). The interviews were semi-structured focusing on medical background, cancer family history taking (attitudes, interpretation, and impact on patient care) and cancer genetics (referral for testing, experiences and educational needs), lasting 45-90 minutes. We report here on responses from the first group of interviews regarding collection and use of family history information. **Results:** To date 18 PCPs have been interviewed (6 rural, 10 suburban and 2 urban). 61% were women and medical school graduation year ranged from 1963-98. Themes from interviews include: PCPs generally regard cancer family history as important but there is no standardized approach regarding content or process. Many PCPs collect limited data using questionnaires and confirm/expand this information in person. Time is an issue in collecting and updating family history. Cancer family history is regarded as most important for decisions about cancer screening; focus is on cancers for which screening is effective (breast, colon, etc). PCPs lack confidence interpreting cancer family histories and few referrals for cancer genetic counseling are made. Providers in urban settings have difficulty getting reliable family history; health care focus is on immediate issues and less on prevention. **Conclusions:** Results from this ongoing study suggest that family history collection focus is largely on data relevant to actions the PCP can confidently recommend (usually cancer screening). Future efforts to educate PCPs will need to focus on collection of accurate and appropriate family history information and clear criteria for referral for genetic counseling. Design of interventions will need to account for PCP focus on the patients immediate needs and the limited time available for each patient visit.

Service user and providers views of appropriate models of clinical genetics service. *L.M. Davies¹, K. Payne², M. McAllister², S. Nicholls², R. MacLeod², H. Middleton-Price², D. Donnai².* 1) HER@M, The University of Manchester, Manchester, United Kingdom; 2) Nowgen, St Mary's Hospital, Manchester, United Kingdom.

A variety of service delivery models exist, with no norm or agreed standard. This study forms part of the Nowgen research programme and aims to elicit users and service providers views about the relevant attributes and process of a service and inform a systematic review of existing service models to develop examples of best practice. Focus groups of user representatives, users and service providers are being conducted to elicit views of what constitutes appropriate and acceptable services. The focus groups were run by a trained facilitator and audio and video-taped. The tapes were fully transcribed. Qualitative analysis with ATLAS.ti software and the constant comparative method was used. Initial results indicate that users described important service attributes as those focussing on individual problems and service providers had a more global view. Examples of attributes that users felt should be provided included: (i) Up to date objective information, provided in a variety of formats tailored to the individual (and family) consulting; (ii) Open access for support and to ask questions, and regular review of the individual and family; (iii) Emotional and practical support to make informed choices; (iv) Advocacy; (v) Hope for the future. Service providers discussed (i) Co-ordination of a variety of screening, health care and social support services to manage the genetic condition; (ii) Counselling and empathy; (iii) Facilitating access to and contact with other people with similar conditions. These results indicate user involvement in the design and evaluation of services is important if user expectations are to be met.

Accounts of `self and `other family relations in genetic counseling for predictive testing. *S.K. Sarangi¹, L. Howell¹, K. Bennert¹, A. Clarke².* 1) Health Communication Research Centre, Cardiff University, Cardiff, United Kingdom; 2) Dept Medical Genetics, Univ Wales Col Medicine, Cardiff, United Kingdom.

Given the familial basis of genetic disorders, clients accounts of decisions about knowing and making known ones genetic status at the stages of decision about testing (DAT) and decision about disclosure (DAD) are rooted in thoughts and potential consequences for other family members. In South Wales, UK, the genetic counseling protocol allows for each clinic session to be a multi-party activity involving not just the individual requesting testing but also other family members. It thus becomes imperative for counselors to find out from the co-present family members what the absent-but-significant others' perspectives might be on the counseling process and outcome. Based on The Wellcome Trust funded project (2000-2004), we analyse 50 audio-recorded and transcribed genetic counseling sessions for HD and HNPCC. Beginning with a demographic map of the participation structure, we identify variations in how self-/other-family relations are constituted in the clinical encounter. Our analytic focus is two-fold. Firstly, we look at how other family members' characters/positionings are talked about in terms of inclusion/exclusion, sharedness/unsharedness, lived histories/future consequences. Secondly, we explore the strategic use of discourse devices such as contrast and reported speech either to endorse or contest various states of affairs. Our data analysis extends the well-established theoretical distinction between 'self' and 'other' to enable a juxtaposition of 'self-family' vs. 'other-family' relational dynamics with regard to the role of partners, siblings, parents etc. At a more specific level we argue that, in the case of DAT, the family relations are articulated using routine accounts (e.g. reproduction, future planning) whereas with DAD, clients' accounting practices are more complex. This complexity can be explained in relation to (1) context-specific familial rights and duties and (2) whether a positive or negative test result is the subsequent outcome.

A randomised trial of the effectiveness of a distraction-based coping intervention for women undergoing cancer genetic risk assessment. *C. Phelps¹, P. Bennett³, K. Brain¹, J. Gray².* 1) Cancer Genetics Service for Wales, University of Wales College of Medicine, Cardiff, United Kingdom; 2) Cancer Genetics Service for Wales, Institute of Medical Genetics, Cardiff and Vale NHS Trust, Cardiff, United Kingdom; 3) Clinical Psychology Department, University of Wales, Swansea, United Kingdom.

Background Studies report that around a third of women experience significant psychological distress when undergoing the early stages of cancer genetic risk assessment, prior to learning their level of risk (e.g. Cull et al, 1999; Fry et al, 2003). Psychological theory and evidence (e.g. Salkovskis & Campbell, 1984; Harvey & Payne, 2002) suggests that cognitive and behavioural distraction techniques may help individuals to control any unwanted intrusive thoughts and reduce distress during this period of waiting and uncertainty.

Method A distraction-based coping leaflet was developed and evaluated through a randomised trial with women referred into the Cancer Genetics Service for Wales. Participants were pre-randomised into either an intervention (distraction leaflet) or control condition (standard information) and completed a postal questionnaire upon referral (T1) and four weeks later, prior to receipt of genetic risk information (T2). 162 participants completed their T1 questionnaires (trial n= 86; control n=76) and 99 participants (n=51 and 48 respectively) completed T2 questionnaires.

Results Analysis of co-variance was conducted using the Impact of Event Scale as the key outcome measure. Amongst women with high levels of distress upon referral, the intervention resulted in significantly lower distress and fewer intrusive thoughts at follow-up than women receiving standard care.

Conclusion The distraction leaflet is an effective intervention for women undergoing cancer genetic risk assessment. The leaflet works by reducing the level of intrusive thoughts about the cancer genetic risk assessment procedure. This intervention may also be appropriate for other aspects of genetic counselling and testing.

The Genetics File: getting the gene into general practice. A. Flouris^{1,2}, C. Gaff^{2,3}, M.A. Aitken^{1,3}, J. Wallace^{1,3}, S.A. Metcalfe^{1,2}. 1) Genetics Education Unit, Murdoch Childrens Res Inst, Melbourne, Victoria, Australia; 2) Dept Paediatrics, The University of Melbourne, Victoria, Australia; 3) Genetic Health Services Victoria, Melbourne, Australia.

A practical genetics educational intervention was developed for general practitioners (GPs)/family physicians in Australia, following a needs assessment responding to greater recognition of the role of genetics in primary care. The intervention includes a resource, available as a printed up-dateable folder, CD-ROM and online, and a highly interactive case-based workshop. Content and format of the resource, The Genetics File, was informed by GPs. It includes sections on: talking with families about genetics (including ethical and counselling issues), familial cancers, haemochromatosis, adult-onset neurological conditions, testing during pregnancy, Down syndrome, fragile X syndrome, cystic fibrosis, newborn screening, glossary, and referral guidelines for familial cancers and clinical genetic services. Content includes information about the condition, which investigations should be performed, family implications, role of GPs in ordering genetic tests and management, when and where to refer, including support groups, and information for families. Resource/workshop content was developed in close collaboration with GPs, specialists and consumers. The educational intervention, attracting maximum Continuing Professional Development points, was rolled-out in July 2003 over 10 months, with a media/mailout campaign targeted to all GP practices in Victoria, and 23 workshops conducted. Evaluation includes a validated questionnaire measuring categories of knowledge, attitudes, skills and behaviour, administered prior to workshop, then 1 and 6 months later. Referrals to clinical genetics services, genetic testing and support groups are also included in the evaluation. These measures combined assess the impact of the intervention on practice of genetic medicine by these health professionals. The mean rating of the intervention by GPs was 8.9 out of 10. Results to date indicate there is a significant improvement in all categories of responses ($p < 0.0001$), which translates to changes in referral practice behaviour.

Virtual labs are the way forward for computer aided Genetics teaching to medical undergraduates: Our experience with VIRTLAB. *P.M. Frossard¹, M.A. Bangash²*. 1) Biological & Biomedical Sciences, Aga Khan University, Karachi, Pakistan; 2) School of Philosophy, University of Leeds, UK.

Virtual (dry) labs are the future of beginner-level medical teaching in Genetics. The high cost and the larger amount of time required in running and maintaining wet labs for medical teaching purposes makes such labs unsustainable in the long run. This is especially the case for medical undergraduates, where only a minority go on to practically employ the experimental skills they learn in medical school. Here we report our experience with VIRTLAB, a virtual laboratory of molecular biology based on the Problem-Based-Learning Pathway (PBL) (Lazzetti et al. *Bioinformatics* 1998; 14 (9): 815-6) VIRTLAB can simulate most of the basic molecular biology techniques: plasmid preparation, restriction enzyme digestion, analytical and preparative agarose gels, DNA cloning and DNA sequencing. We used VIRTLAB to supplement our lectures on Genetics to 2nd year Medical Undergraduates at the Aga Khan University, where we have recently initiated a PBL based curriculum. We designed clinically-oriented case studies involving prenatal diagnosis of a genetic disorder; here the exercises required the students to design experiments and run diagnostic tests on VIRTLAB simulations. A questionnaire delivered after the lab asked students to rate their responses; most found the lab to be highly interactive (79%), simple (75%), fast (86%), providing good feedback (61%) and simulating an actual laboratory well (52%). The overall effectiveness was on a highly favourable scale (81%). VIRTLAB will be of little benefit to those planning long-term career in Genetics research or as physician-investigators. However, its use for medical undergraduate teaching cannot be overemphasized; for this particular cohort, our model of virtual teaching calls for a greater emphasis on dry laboratories and computer simulations together with a sound theoretical background. Our subsequent experience with VIRTLAB and other virtual tools will undoubtedly shed more light on this exciting development.

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The North West Genetics Knowledge Park (Nowgen)-Engagement with Genetic Support Groups. *H.R. Middleton-Price¹, D. Donnai², J. Ayres¹.* 1) Nowgen, St Mary's Hospital, Manchester, United Kingdom; 2) Regional Genetics Service, St Mary's Hospital, Manchester, United Kingdom.

Central to Nowgen's core work plan is an active programme of engagement with genetic service users. The first Nowgen Support Group Evening was held in April 2004 and is one of a number of innovative approaches adopted to promote dialogue between Nowgen and support group representatives, as well as to facilitate networking opportunities between these groups. We have developed a database of genetic support group information, and used this to facilitate initial contact. The Nowgen Support Group Database will be placed on the Nowgen website, currently under construction. A total of 128 introduction letters were sent and the event was also promoted on several websites and in support group publications. In total 57 came to the event, with 47 of the attendees representing support groups. The event was held in the evening and the programme included some brief introductory talks followed by a buffet and networking session. Twenty one evaluation forms were completed. The overall feedback was extremely positive. A large majority of participants stated that they wanted to have regular similar meetings and be actively involved in the Nowgen Centre. Nearly all respondents want to contribute to the Nowgen Newsletter and have representation on the Nowgen Website. Many appreciated the opportunity to meet together and discuss common issues with representatives of different groups. Nowgen is using this feedback to inform and direct our next steps in our active user and public engagement programme.

Genetic risk calculations for cystic fibrosis in neonatal screening by immunoreactive trypsinogen and CFTR

tests. *B. Gold*¹, *R.B. Wilson*², *P. Flodman*³, *W.W. Grody*⁴, *S. Ogino*^{5,6,7}. 1) Laboratory of Genomic Diversity, National Cancer Institute, Frederick, MD; 2) Dept. of Pathology and Lab. Med., University of Pennsylvania, Philadelphia, PA; 3) Dept. of Pediatrics, University of California Irvine, CA; 4) Depts. of Pathology and Lab. Med., Pediatrics, and Human Genetics, UCLA School of Medicine, Los Angeles, CA; 5) Dept. of Pathology, Brigham and Women's Hospital, Boston, MA; 6) Dept. of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA; 7) Harvard Medical School, Boston, MA.

Risk assessment is an essential component of genetic counseling and testing, especially for cystic fibrosis (CF), the most common severe autosomal recessive disease. Despite controversy, neonatal or newborn screening for CF is widely practiced commonly by immunoreactive trypsinogen (IRT) test followed by CFTR test if IRT test is positive. However, systematic methods for accurate risk calculations are currently lacking. Risk assessment is particularly problematic when IRT test is positive and only one CFTR mutation is detected. We meta-analyzed published data to evaluate assay characteristics of immunoreactive trypsinogen test. The probability that a neonate has positive IRT test, if the neonate is affected, a carrier or a non-carrier, is estimated to be ~1, 0.04, or 0.011, respectively. We provide methods to calculate genetic risks for a variety of commonly encountered scenarios. Our scenarios include neonates with mixed ethnicity, neonates with a family history of CF, and neonates of parents with genetic test results. Our Bayesian methods permit CF disease probabilities to be calculated accurately, taking into account all relevant information.

Bayesian risk calculations for cystic fibrosis: avoiding pitfalls in complex scenarios. *S. Ogino*^{1, 2, 3}, *R.B. Wilson*⁴, *B. Gold*⁶, *P. Hawley*^{3, 5}, *W.W. Grody*⁷. 1) Dept Pathology, Brigham & Women's Hosp, Boston, MA; 2) Dana-Farber Cancer Institute, Boston, MA; 3) Harvard Medical School, Boston, MA; 4) University of Pennsylvania, Philadelphia, PA; 5) The Children's Hospital, Boston, MA; 6) National Cancer Institute, Frederick, MD; 7) UCLA School of Medicine, Los Angeles, CA.

INTRODUCTION: Risk assessment is an essential component of genetic counseling and testing, and Bayesian analysis plays a central role in complex risk calculations. We have previously developed generalizable Bayesian methods to calculate the autosomal recessive disease risk of a fetus when one or no mutation is detected, and another independent risk factor is present. Our methods are particularly useful to calculate the CF disease risk for a fetus with echogenic bowel. In practice of genetics, however, there are many other scenarios that cannot be calculated by our previous methods. **METHODS AND RESULTS:** We herein provide methods of calculating genetic risks for a variety of clinical settings that are rather common. Our scenarios include: different mutation panels have been used for the parents and for a fetus; genetic testing results are available on the proband or other relatives, in addition to the consultand; fetal ultrasound is negative for echogenic bowel while a family history of CF is positive; and the consultand has mixed ethnic background. **CONCLUSION:** Our versatile Bayesian methods are applicable to many different common scenarios, and that allow the autosomal recessive disease and carrier probabilities to be calculated accurately, taking into account all relevant information. Our methods will provide accurate genetic risk estimates to patients and their family members for CF or other autosomal recessive disorders.

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HISTORY OF HUMAN AND MEDICAL GENETICS. *P. Harper.* Inst Medical Genetics, Univ of Wales Col Medicine, Cardiff, United Kingdom.

The history of our rapidly changing field will be seen in later years as one of the most important parts of the history of science and medicine. Since much of it is within reach of living memory, we have a unique opportunity to document it fully, but this will be lost if we do not take it rapidly. An international initiative is presented here which aims to encourage and link both geneticists and historians active and interested in historical aspects and to complement those activities already underway. Work in progress includes:- Expert archiving of scientific records of retired or deceased workers; A Human Genetics Historical Library of important books; International workshops to bring together those interested; Audio-recordings of interviews with key workers; A regular electronic newsletter and website (genmedhist.net); A linked structure for historical interest groups in different countries. It is hoped that these and other activities will help to ensure that the primary historical material underlying human and medical genetics will be preserved and made available for all those wishing to undertake more detailed studies.

SKELNET - German skeletal dysplasia network as a national interactive telemedical diagnostic and research approach to rare diseases. *S. Despres¹, O. Hiort², K.H. Klingebiel², A. Rauch², A. Reis², A. Winterpacht², K. vonderMark², A. Vortkamp², D. Haas², A. Superti-Furga², M. Hrabe de Angelis², M. Briggs², R. Schumacher^{1,2}, J. Spranger^{1,2}, B.U. Zabel^{1,2}.* 1) Molecular Genetics , University Childrens hospital, Mainz, Rhld.Pf., Germany; 2) SKELNET group.

The skeletal dysplasias are a clinically and genetically extremely diverse group of genetic diseases primarily affecting the development of the skeleton. The rarity, extensive heterogeneity and complex pathophysiology have made the skeletal dysplasias a challenge to study. Recently a pan-European integrated diagnostic and research network for skeletal dysplasias (ESDN, www.esdn.org) was established. Key features of the pilot project are (1) the diagnostic aspect focused on providing and evaluating an integrated clinical/radiological and DNA diagnostic network and (2) a coordinated research aspect focused on identifying the genes, mutations and disease processes that underlay skeletal dysplasias. This type of network has not previously been attempted and therefore the success of the project will have major implications for the delivery of future diagnostic services for rare genetic diseases. In the meantime ESDN will be complemented by the German skeletal dysplasia network SKELNET which will further concentrate on telemedically based X-ray composition and interchange for the complete medical patient care process. The SKELNET-server is an internet based server providing DICOM-PACS and patient records even through firewall protected environments. Radiographs or other media are assigned to a patient record, whereas personal data are stored and transmitted with state-of-the-art data protection. Experts can collect cases of certain diseases, can assign their lab data to the cases and can initiate consultations with SKELNET members in a safe way. Outpatient offices can send their data (patient information, ultrasound images, X-rays, even videos) to request help or continued care. SKELNET technology combines networking, knowledge collection, PACS and patient record database technology (RDE), internet security technologies and patient data protection mechanisms to an optimal rare disease collection, analysis and care workhorse.

Detection of large pathogenic *SCA2* and *SCA7* expansions by repeat-primed PCR assay. C. Cagnoli¹, G. Gerbino Promis², C. Michielotto², G. Stevanin³, M. Viemont³, E. Dragone², C. Gellera⁴, N. Migone^{1, 2}, A. Brusco^{1, 2}. 1) Dept Genetics, Biol & Biochem, University of Torino, Italy; 2) S. Giovanni Battista Hosp., Medical Genetics Unit, Torino; 3) INSERM U289, IFR70 & Dept Genetics, Salpêtrière Hosp. APHP, Paris, France; 4) Dept. Biochemistry & Genetics, Neurological Institute. C.Besta, Milan, Italy.

The CAG repeat in the *SCA2* and *SCA7* genes can expand above 100 repeats causing infantile and juvenile-onset spinal cerebellar ataxia (SCA). Routine assays for *SCA2* and *SCA7* based on fluorescent or silver-stained PCR, can not reliably detect such large expansions. We developed a fluorescent STR-primed PCR based on a labelled forward locus-specific primer, and a reverse primer complementary to the CAG repeat. We tested: 35 *SCA2* normal subjects (22-34 CAG) and 12 affected (10 in the range 37-53 CAG, one 23/200, and one 22/350, kindly provided by Molecular Genet. Lab., Mayo Clinic, USA); 39 *SCA7* normal subjects (9-34 CAG) and 26 affected (36-106 CAG). In case of an expansion, the pattern was characterized by an array of gradually descending spikes, that contained a bell shaped array of peaks in the right end of the profile whenever the expanded allele was small. Routine-PCR in our laboratory could not identify *SCA2* large expansions (200 and 350 repeats), or *SCA7* alleles above 80-CAG, whereas STR-primed PCR detected both the two *SCA2* large expanded patients and all *SCA7* expansions (up to the largest available of 106 repeats). STR-PCR, however, does not allow to infer the precise number of repeats in the expansion; small expansions in the borderline range around 36-40 repeats, were not discriminated from normal alleles in the upper side limit (>30 CAG). We conclude that this technique might be a valuable tool to identify expansions above 40 CAGs in the *SCA2* and *SCA7* genes, without false-positive or false-negative results. We think the test, easy to set-up and very reproducible, could be of practical value in the prenatal diagnosis offered to affected or pre-symptomatic at risk parents, or in routine screening for infantile or congenital hypotonia associated with non-specific neurological symptoms, a possible early-onset *SCA2* or *SCA7* disease.

Evaluation of paternal family history of breast cancer in primary care. *J. Culver^{1,2}, S. Hall², N. Press³, L. Pinsky¹, S. Reynolds¹, W. Burke^{1,2}.* 1) University of Washington, Seattle, WA; 2) Fred Hutchinson Cancer Research Center, Seattle, WA; 3) Oregon Health and Science University, Portland, OR.

Overlooking paternal family history in breast cancer risk assessment may lead to underestimation of risk and inadequate preventive care. This study aimed to determine whether primary care physicians identify a paternal family history of breast and ovarian cancer and how such history influences patient care. We evaluated providers using concealed standardized patients (SP's) trained to present concerns and family history in a consistent way. The study included 86 internal medicine or family practice physicians in the Seattle area who consented to an unannounced, audio-taped SP visit. Physicians were randomized to receive one of three SP cases. We present results from 33 physicians who saw Rachel, a 36-year-old with a paternal family history of cancer. Rachel presents to establish care and renew a prescription for her stress-induced migraines, which were recently aggravated by the diagnosis of breast cancer in her 48-year-old sister. Unless asked, she does not reveal her paternal family history, including an aunt with breast cancer at age 42, an aunt with ovarian cancer in her 40s, and a grandmother with a female cancer. Analysis of visits revealed that 15/33 physicians identified her aunt with breast cancer, but only 2/33 identified all affected relatives. While 32/33 indicated Rachel has an increased breast cancer risk, only 2/33 communicated a significantly increased risk. Additionally, 8/33 discounted her genetic risk either because affected relatives are on her father's side or because her mother is unaffected. The majority (23/33) suggested she initiate mammography screening in the near future. When asked about genetic testing, only 1/33 indicated she is a candidate for testing and 3/33 discussed a genetic counseling referral. Thus, most physicians did not take sufficient family history to identify a high risk, some did not recommend early mammography, and few identified Rachel as a candidate for a genetics referral. These results indicate that paternal family history may be underappreciated in primary care, identifying an area of opportunity for physician education.

Challenges in assessing the clinical validity of BRCA1 testing. *J. Tranchemontagne¹, L. Boothroyd¹, J. Joyce², I. Blancquaert¹.* 1) Agence d'évaluation des technologies et des modes d'intervention en santé Montréal, PQ, Canada; 2) Canadian Coordinating Office for Health Technology Assessment Ottawa, Ontario, Canada.

In preparation for a health technology assessment report on molecular testing for hereditary breast and ovarian cancer (HBOC), the literature was reviewed to assess clinical validity. The evaluation of molecular genetic tests poses the challenge of choosing a gold standard. While the definition of HBOC relies on family history, there is currently no consensus on the minimal risk factors defining a HBOC family. This paper describes the challenges encountered while assessing the clinical validity of BRCA1 testing for HBOC and how it relates to penetrance and genetic heterogeneity. Method: Published and grey literature was identified in January 2003 for 1999 on (updating in July 2004) by searching PubMed, Cochrane Library, MEDLINE, CANCERLIT, EMBASE, and Biosis Previews, the Internet, trial registries, guidelines databases, and the websites of health technology assessment agencies. Results: Difficulties encountered included defining the HBOC phenotype, gaps in knowledge regarding mutation distribution, variations in the selection of individuals and techniques used for testing, as well as the nature of the available evidence and the means of reporting. Clinical sensitivity data was extrapolated from studies designed for other purposes, taking into consideration the potential misclassification of results for families with negative results following all mutation screening strategies. Estimates of the clinical sensitivity of exon and intron/exon junction screening techniques in BRCA1 were derived by examining the proportion of HBOC families carrying point mutations / small rearrangements versus those with large genomic rearrangements / non-coding region mutations. Clinical sensitivity values were expressed as a range of values. Conclusion: It is worthwhile pursuing research on clinical validity, in order to increase the precision of clinical sensitivity estimates, for instance. Future studies should, however, use sound methodology and clear selection criteria for the population of interest.

Colorado's First Adult Medical Genetics Clinic. *M. Taylor, L. Ku, J. Feiger.* Adult Medical Genetics Program, University of Colorado Health Sciences Center, Denver, CO.

Background: Adult clinical genetics is considered an expanding field, but currently there are few clinics dedicated to adult genetic conditions. We developed a general adult medical genetics (AMGP) clinic targeting all adult genetic conditions with the exception of hereditary cancers. The AMGP clinic is unique in Colorado and is supported entirely by our Department of Medicine. Here, we report on the structure and productivity of our AMGP clinic during its first 2 years of operation. **Results:** The clinic is staffed by one genetic counselor and one medical geneticist/internist. The main indications of referral consist of general genetics/dysmorphology (45%), connective tissue disorders (20%), neurogenetic conditions (19%), and neurocutaneous conditions (11%). 51% of patients have a personal history of a genetic condition, 22% a personal and family history, 24% a family history alone, and 3% were seen for population screening. New patient visits steadily increased in the first year, from only 4 visits/month in the first three months of operation to 15 new visits/month in the 9-12th months of operation. The majority of the new patient visits were new to our hospital system, indicating a heavy source of outside referrals; increasing internal referrals remains a challenge. Hospital policy does not permit institutional billing for genetic tests; therefore, genetic tests must be billed to the patient or to insurance directly. 45% of genetic tests were ordered from the in-house DNA Diagnostic Laboratory and 55% from outside laboratories. 8% of the patients elected to self-pay for the genetics visit; usually for privacy issues (rather than lack of insurance coverage). 59% of patients were insured by managed care. 21% of patients were insured by Medicare or Medicaid. **Conclusions:** In the two years since inception, our clinic has grown in terms of our referral and patient base as well as with the diversity of genetic diseases seen. Significant challenges remain in terms of obtaining financial support from our hospital, in facilitating genetic testing for our patients, and increasing visibility of the clinic services to the general community.

Advocacy Organization Owned Genetic Alliance BioBank is a Catalyst for Research. *E.W. Johnson^{1, 2}, G. Mason^{2, 3}, P.F. Terry², J. Scott², S.F. Terry².* 1) PreventionGenetics, Marshfield, WI; 2) Genetic Alliance BioBank, Washington, DC; 3) Inflammatory Breast Cancer Research Foundation, Bainbridge Island, WA.

Advocate owned research foundations represent the thousands of people affected by genetic conditions. These groups seek the tools that will enable them to characterize the range of the phenotype of the condition, rather than extremes, leading to earlier and better clinical methods of detection and diagnosis, with the ultimate goal of finding an effective treatment. Genetic disease research is often hampered by the lack of an easily accessible, linked, well-defined patient cohort. For rare conditions, existing resources are often fragmented, "competitively isolated" and under-funded. The Genetic Alliance BioBank (GAB) was established to help remove some of these barriers to effective research. The GAB enables advocacy organizations to partner in a comprehensive BioBank to facilitate disease specific research while sharing resources and expenses to accomplish what each group could not feasibly do alone. GAB provides high quality, centralized, standardized collection and archiving. Each advocacy organization has exclusive control over their samples and data. All member organizations use GAB IRB approved, standardized forms. Individual donors for the BioBank are recruited by the advocacy organizations in an atmosphere of support and trust following a culturally sensitive, comprehensive, informed decision-making process. Sample and information access for the research and clinical communities are facilitated significantly by the advocacy organizations. For the advocacy organizations involved, proven results include a role in gene discovery, mutation database development, and numerous research projects while maintaining rigorous participant protection. The involvement of the various disease specific advocacy organizations allows cross-disease research and unique collaboration. Ethical recontact and longitudinal studies are both part of this model. This new paradigm brings individuals and their organizations into a true partnership with researchers, ensuring the benefits outweigh risks and helps accelerate the translation of research.

Challenges with the uptake of nanotechnology into medical genetics. *G. Khushf¹, R. Best²*. 1) Center for Bioethics, University of South Carolina, Columbia, SC; 2) University of South Carolina, School of Medicine, Dept. Ob/Gyn, Columbia, SC.

Considerable attention has been given to the development of nanotechnology and the many practical applications it might bring including a wide range of tools for medicine. Federal research funding has increased dramatically for nanotechnology in recent years, and NIH has announced the development of programs in nanomedicine with major Nanomedicine Center funding beginning in 2005. Within the community of nanoscience researchers there is a belief that much of the fundamental work has transitioned to the application end, and indeed, much of the research is at the place where it could be applied. However, there is a significant disconnect between the basic science community and those providing clinical care. The disconnect is well exhibited in the area we explore in this presentation; namely the use of quantum dots and their potential use in clinical molecular cytogenetics. We address some of the conceptual, cultural, and institutional conditions that are critical for the transitioning of an emergent technology into the clinical setting. After exploring the challenges that have been associated with applying quantum dots into molecular cytogenetic testing, we provide recommendations for the development of trading zones between scientists and those in clinical practice so that there might be a more rapid transition from cutting edge research into clinical practice.

Use of a novel computer program to assess family history. *K.D. McKelvey Jr¹, J.P. Evans², J. Emery³.* 1) Dept of Family and Preventive Medicine, University of Arkansas for Medical Sciences, Little Rock, AR; 2) Dept of Genetics, University of North Carolina, Chapel Hill, N.C; 3) Discipline of General Practice, University of Western Australia.

The purpose of this study was to determine the feasibility of using GRAIDS (Genetic Risk Assessment on the Internet and Decision Support) software as a screening tool for assessing hereditary cancer risk by family history in an office setting. Advances in cancer genetics have the potential for significant impact in primary care, but the application of these advances in the context of a busy medical practice is an enormous challenge. Genetic testing for cancer susceptibility involves time-intensive counseling prior to testing, carries with it unique psychosocial issues, and population-based genetic screening is not feasible or appropriate. Therefore a screening method is necessary to identify patients at elevated hereditary risk who may benefit from individualized surveillance strategies and further genetic consultation. Family history is often the most significant risk factor for cancer and is a useful screening tool but is underutilized in preventive healthcare. GRAIDS software (a proprietary software package from AP Benson) was used to help obtain family history and assess risk in a primary care practice. Patients in the waiting area at an outpatient primary care facility were offered the opportunity to participate in this study by means of a notice posted at the check in desk. Over a 20 work day period, 71 patients out of an estimated 800 chose to use the program and provide their family history pertinent to cancer to a medical professional (KM) trained in the use of GRAIDS software. Of the participating subjects, 7 (10 percent) met predetermined eligibility for further genetic referral. The average length of each session was 18 minutes. The data indicate that it is feasible to obtain and enter a targeted three generation family history into a computer program and discuss inherited cancer risk during the course of a routine office visit. This study provides a necessary step toward establishing the feasibility of automating family history risk assessment.

Program Nr: 1459 from the 2004 ASHG Annual Meeting

Craniofacial Brazil project: access for genetic evaluation and genetic counseling in the Reference Net for Craniofacial Treatment. *V.L. Gil da Silva Lopes¹, I.L. Monlleo²*. 1) Genética Médica, UNICAMP, Campinas, São Paulo, Brazil; 2) Pediatria, FM/UNICISAL, Alagoas, Maceió, Brazil.

This present study is part of the Craniofacial Brazil Project, which is the first initiative for national characterization of the health care in craniofacial area in Brazil. The majority of the affected individuals has been treated in one of the 29 public specialized centers, in a Reference Net for Craniofacial Treatment, which was created in 1998. In order to characterize the access for genetic evaluation and genetic counseling for individuals in this centers, it was used a patterned questionnaire, by mail. Among the 13 centers with clinical geneticist, 61,8% had one professional and were situated in Universities, most of them in the state of So Paulo. In spite of few clinical geneticists in the composition of the teams, 22 centers referred that patients and parents asked for etiology and prevention. These informations were given in 80% of the centers and in 55% of them by non-specialized physicians. The clinical geneticist is involved in recognize, notified and registry congenital defects, as well as in the determination of natural history, anticipatory management of complications and genetic counseling for the family and the proband, in the future. The absence of this professional certainly makes uncompleted the follow up of craniofacial patients and families. This results suggested that the access for clinical geneticist is not enough and it would be necessary the revision of parameters for inclusion of this specialist in craniofacial centers teams. Also these data suggested the need to establish the better health policy for CFA individuals in our country. E-mail: cranface@fcm.un.

Student Screening for Genetic Blood Diseases in Bahrain (1999-2004). *S. Al Arrayed.* Genetic Dept, Salmaniya Medical Ctr, Manama, Bahrain.

The inherited disorders of hemoglobin is common in Bahrain. The student screening project had been initiated in 1999 and continues for the subsequent six years 2000-2001-2002-2003-2004. We are presenting this project and comparing the results of these six years. The aim is to raise awareness among the students about hereditary anemias, and determine prevalence among them. **Material and Method:** The plan was to screen all the students in the 11th grade (2nd Secondary). The project includes education sessions, blood collection, laboratory testing, data processing, distribution of cards, data analysis, and reporting. Permission for screening was taken from parents, and the response rate was 80-85% every year. The students were fully informed about the diseases through educational sessions. The total number of students screened was 5685, 5677, 6978, 5883, 5410, 6228 students respectively. The total number of students screened until now is approximately 36,000 students. Technicians visited the school to withdraw the blood samples and these were tested on the same day. High pressure liquid chromatography (HPLC machine) was used to perform the hemoglobin electrophoresis. The blood samples were also tested for, G6PD deficiency testing. A coded form was designed with the demographic information of each student and included the results of the tests. At the end of the campaign each student received a card with the lab results. **Results:** Comparing the prevalence results during the years 1999-2000-2001-2002, 2003-2004 The study showed that the Prevalence rate of Sickle cell disease among these students was 1.2%, 1.3%, 1.09%, 1.09%, 1.3%, 1.25% respectively. Sickle cell trait was found in 13.8%, 13.8%, 14.2%; 14%; 13%, 14%, respectively. While Beta thal trait was found in 2.9%, 3.6%, 2.6%, 3.7%, 2.9% respectively, and G6PD in 23.3%, 23.4%, 21.9%, 20%, 20.7%, 23%; respectively. The study included reports on the prevalence of these diseases by region, and by school. It also showed the level of hemoglobin S, F, A₂, and H in each group. **Conclusion:** Preventive measures such as health education, carrier screening and premarital counseling remain the best ways of dealing with these diseases. As prevention can result in significant financial saving, social and health benefits.

Program Nr: 1461 from the 2004 ASHG Annual Meeting

A model for outreach and education for Ashkenazi Jewish Genetic diseases in a large Jewish community. A. Schneider, L. Gross, D. Dorsainville, R. Keep. Dept Genetics, Albert Einstein Medical Ctr, Philadelphia, PA.

The Ashkenazi Jewish (AJ) community in Philadelphia is spread out over a large geographic area. Outreach and education are made more difficult in that many members of this community are not affiliated with religious centers and do not strongly identify themselves as Jewish. It is estimated that 1 in 4 individuals of AJ ancestry is a carrier of 1 of the 8 diseases more common in this community (Tay-Sachs, Canavan, Gaucher, Niemann-Pick, Bloom, Fanconi, Familial Dysautonomia and Cystic Fibrosis); thus we would expect to find about 50,000 carriers among the approximately 200,000 Jewish individuals in Philadelphia and its adjacent counties. The Ashkenazi Jewish Genetic Disease Screening Program at Albert Einstein Medical Center in Philadelphia was established in 1999. The Program provides outreach, education, genetic counseling and carrier screening to individuals of Ashkenazi Jewish descent. Despite extensive efforts to educate the public and physicians, the overall interest from the Jewish Community has been disappointing. To date we have screened about 500 individuals. Of these individuals, 85 are carriers of one disease, 9 are carriers of 2 diseases and 1 has Gaucher disease and is a carrier of another disease. Overall approximately 1 in 5 is a carrier of one of these diseases. We concluded that hiring an outreach coordinator to help focus our efforts on our most responsive groups—engaged couples, married couples with no children, and college students—would help to increase uptake of testing. We will discuss how the addition of an outreach coordinator has enhanced our ability to reach the community in general and college students and newlyweds in particular.

Awareness of family history of cardiovascular disease: Implications for public health. *M.T. Scheuner^{1, 2}, W.C. Whitworth², P.W. Yoon², M.J. Khoury².* 1) Dept. of Health Services, UCLA School of Public Health, Los Angeles, CA; 2) Office of Genomics and Disease Prevention, CDC, Atlanta, GA.

Family history tools can improve risk assessment for cardiovascular disease (CVD). For these tools to be effective the public must be aware of the disease status of their close relatives. We examined awareness of family history of coronary heart disease (CHD) and stroke among 4035 respondents to the HealthStyles 2003 national survey. We asked about each respondent's mother, father, siblings, mother's relatives and father's relatives. Response options for CHD and stroke were: yes diagnosed at or before age 60; yes diagnosed after age 60; no; and don't know. Awareness of disease status was gauged by the frequency of don't know responses. Respondents were 60% female, 72% white, and mean age was 48.4 yrs (SD 14.4 yrs, range 19 to 93 yrs). There were 257 (6%) with personal history of CHD and 171 (4%) with stroke. The proportion that did not know about CHD and stroke for mothers was 7.6% and 6.2%, respectively, compared to 11.4% and 10.5% for fathers. The proportion that did not know about CHD and stroke for mother's relatives was 31.7% and 32.0%, respectively, compared to 38.4% and 36.9% for father's relatives. Logistic regression analysis assessed the influence of personal factors on awareness of disease status in relatives. Age, personal history of CHD, education and income were highly significant factors associated with awareness of CHD in relatives ($p < 0.0001$); sex and race were not. Results were similar for stroke; however, personal history of stroke showed a weaker association ($p=0.02$) and sex was weakly associated ($p=0.02$). In conclusion, most individuals can report a status of CHD and stroke in their relatives, particularly their first-degree relatives. There is more awareness about maternal relatives. Age, education, income and personal history of disease are significant factors that influence awareness of CVD status in relatives. Understanding these differences through further study could lead to targeted educational programs that increase awareness about the importance of family history, which may ultimately improve familial risk assessment and prevention efforts.

Program Nr: 1463 from the 2004 ASHG Annual Meeting

Genomic tools for the diagnosis and evaluation of mental retardation: emerging legal and ethical issues. *M. Lacroix, B.M. Knoppers.* CRDP, Faculte de droit, Universite de Montreal, Montreal, Quebec, Canada.

The inclusion of vulnerable populations such as children and persons with disabilities in research studies has received special protection since the adoption of the Nuremberg Code. More recently, the need to include children in research has gained recognition, as has the need to guard against group harms in human genetic research involving ethnic groups or sub-populations. This presentation will use the *Genomic Tools for Diagnosis and Evaluation of Mental Retardation* project as a case study to illustrate the ELSI issues that may arise in research related to DNA sampling and vulnerable populations. This multi-centre research project aims to evaluate high-resolution BAC microarray comparative genomic hybridization as an alternative method of identification of chromosomal abnormalities in people with mental retardation. It will obtain patient data and specimens through the Canadian Molecular Cytogenetics Research Consortium, a group of clinical geneticists and cytogeneticists throughout Canada, and will compare samples from affected individuals and their family members with those from healthy adults. The project raises a number of ethical, legal and social issues which will also be examined by the research team, such as: the implications of singling out children with mental retardation for study, the implications for, and the rights of, other family members, the implications for health care services or counselling, and the involvement of ethnically diverse healthy adults in genetic variation studies unrelated to the presence or absence of disease.

Program Nr: 1464 from the 2004 ASHG Annual Meeting

The Iranian Human Mutation Database. *M. Motazacker*¹, *E. Taherzadeh*¹, *Z. Hussein*², *M. Kamyab*², *H. Najmabadi*¹. 1) Genetics Research Ctr, USWR Univ, Tehran, Iran; 2) Tebyan IT institute, Tehran, Iran.

Regional genetic databases provide scientists all over the world with a valuable resource of specific information about the genetic structure of a particular region in order to promote diagnosis, treatment and basic research on genetic disorders. Iranian population consist of different races, tribes and religions has a highly heterogeneous gene pool and mutation spectrum which in many cases can also be extrapolated to the mutation spectrum in the neighbouring countries in the middle east. The Iranian Human Mutation Database (IHMD) established in February 2004, is the collection of the information about published or submitted mutations and related polymorphisms from Iranian population and is created to facilitate accessing to such a great resource of genetic data. IHMD is accessible through World Wide Web using url: <http://www.uswr.ac.ir>. Up to know more than 392 mutations related to 95 genetic diseases with distinct gene have been submitted with the information about mutation(s) or polymorphism of each gene. References and authors are also listed by the mutation.

Program Nr: 1465 from the 2004 ASHG Annual Meeting

Iranian Human Mutation Gene Bank. *M. Neishabury, Y. Shafaghati, M. Motazacker, K. Kahrizi, M. Jalalvand, S. Bany Hashemi, B. Moghimi, A. Noorian, S. Mosayyebi, K. Javan, H. Najmabadi.* Genetics Res Ctr, Univ Of SWRS, Tehran, Iran.

With the increasing number of disease causing genes identified and the existence of genetic variation between different populations in the world, establishment of population-specific DNA banks could provide valuable sources of information and sample for medical genetic research in the field of diagnosis, prevention and hopefully cure of hereditary diseases world-wide. Iran, a country with nearly 70 million population, represents a highly heterogeneous gene pool and mutation spectrum due to the unique nature of its geographical, cultural and ethnical diversity. The data base of Iranian Human Mutation Gene Bank is a comprehensive source of information on DNA samples collected in this bank during the last 6 years. The samples belong to the patients with genetic disorders with mendelian mood of inheritance studied in Iran. Some of the samples are assigned to common or novel mutations and some others belong to patients with clinical profiles associated with particular genetic diseases but unidentified mutation. The new version of the software presents different categories of genetic disorders including Hemoglobinopathes, Neuromuscular disorders, Mental Retardations and Hearing loss. With the exception of personal data, which is strictly kept confidential, clinical profile for each individual, and genetic information including pedigree (if available) for each family is presented in this data base. In order to facilitate collaboration with other scientists in the world with the same interests, we also display the information regarding our experimental projects at this center on some of these genetic disorders. This DNA bank offers a free of charge sample resource from a large heterogeneous population to all the scientists in the world, who are working on the various aspects of genetic disorders from prenatal diagnosis to gene structure and function. No commercial benefit is involved in establishment of this DNA bank.

Program Nr: 1466 from the 2004 ASHG Annual Meeting

The Parkinson disease mutation database (MutPD). *N. Pankratz, T. Foroud.* Medical & Molec Genetics, Indiana Univ Sch Medicine, Indianapolis, IN.

In the past six years, scientists have identified seven genes that have been shown to cause Parkinson disease (PD). As more and more individuals with PD are tested for mutations in these genes, it is imperative that their significance is properly understood. This is particularly relevant for the *parkin* gene, in which almost 100 unique mutations have been identified. Already population studies have found several of these mutations in roughly equal frequency among patients and controls. Since genetic counseling should be required with all genetic testing, the presence of non-pathogenic polymorphisms creates a formidable challenge in interpreting the significance of any genetic variation. The Parkinson disease mutation database (MutPD) aims to collect all known mutations and non-pathogenic coding variations in the genes related to PD. With this data in a centralized location, scientists and genetic counselors alike can use the data to better interpret the significance of a mutation. This database will also lay down the framework for an understanding of genotype-phenotype correlations. The MutPD website was launched in July 2004 and is being updated continuously.

Program Nr: 1467 from the 2004 ASHG Annual Meeting

Relational Database System for Management of Molecular Diagnostic Laboratory Services. *Z. Rennert, C. Sadowl, D. Nielsen, D.G.B. Leonard, V. Van Deerlin, H. Rennert.* Path. and Lab. Medicine, University of Pennsylvania, Philadelphia, PA.

We have designed and developed a modular relational database system for managing molecular diagnostic data from all stages of molecular testing using Microsoft ACCESS. The system resides on a shared drive, is pass-word protected and can be accessed via the laboratory net work. The system allows rapid storage and tracking of data including demographic, clinical, specimen description and test results information in a longitudinal fashion. For simplification and standardization, most of the data is accessed using pull-down menus. All information pertaining to specimen, tests ordered and test results is coded via set up screens and the values are selected via look-up tables. All data in all tables that pertain to any particular patient are linked together by a unique identifier. Extensive search screens are provided for locating patients, specimens, tests ordered and results previously logged into the system. Test results are entered into test-specific screens tailored specifically for the information required for each test. Statistical reports are provided via Excel spreadsheets and Excel Pivot tables can be generated for easy data manipulation. The database is easy to use and useful, greatly improving data management, quality assurance and patient care in our laboratory. In addition, the modular design allows modification and expansion of the database to accommodate special studies.

Identification of Knowledge and Misinformation Associated with Uptake of *BRCA1/2* Testing Among Community Women with Early Onset Breast Cancer. *J. Stopfer*¹, *N. Peters*², *R. Polis*², *A. Rose*², *S.M. Domchek*^{1,2}, *K. Armstrong*^{1,2,3,4}. 1) Abramson Cancer Ctr; 2) Department of Medicine; 3) Center for Clinical Epidemiology and Biostatistics; 4) Leonard Davis Institute; University of Pennsylvania.

Women identified with mutations in *BRCA1/2* can benefit from interventions shown to lower risk and enhance early detection. There has been a lower than expected level of uptake among individuals with a significant chance of having a *BRCA1/2* mutation. Limited information is currently available about how knowledge deficits and misinformation are associated with testing uptake. Here we have investigated the association between attitudes, knowledge and use of *BRCA1/2* testing among women with early onset breast cancer.

A mailed survey was sent to members of two Mid-Atlantic support groups. Knowledge, attitudes, use of *BRCA1/2* testing and sociodemographic characteristics were measured. Of 2400 mailed questionnaires, 493(21%) were returned. 406 respondents had a diagnosis of breast cancer, of whom 248 were diagnosed prior to age 50 and included in the analysis. 83.1% (206/248) of these women had heard of *BRCA1/2* testing yet only 12.5%(31/248) had undergone testing. Women who had undergone testing had significantly higher knowledge than women who had not undergone testing ($p=0.02$) with the largest differences seen for knowledge about the risk of ovarian cancer, paternal transmission of mutations and the prevalence of *BRCA1/2* mutations. Women undergoing testing were younger ($p=0.03$), more likely to have a college education ($p=0.03$), more likely to have a family member who had undergone *BRCA1/2* testing ($p=0.005$), and had more positive and fewer negative attitudes about *BRCA1/2* testing ($p=0.004$ and $p=0.004$).

In this sample of women, lack of information regarding how genetic testing might alter health care decisions (particularly with regard to ovarian cancer risk) and fear about the genetic testing procedure, its costs and the possibility of false positive results were associated with low uptake of genetic testing. Further education regarding these specific points may enhance the use of this genetic testing.

Cancer Genetic Testing and Genetic Discrimination: A Pre-Cancer Risk Counseling Survey of Patient Knowledge and Opinions. *K. Banks¹, J.N. Weitzel², A. Metzenberg¹, V. Vandergon¹, K.R. Blazer².* 1) California State University at Northridge, Northridge, CA; 2) City of Hope, Duarte, CA.

Approximately 5-10% of all cancer is associated with a single-gene inherited trait. For many familial cancer syndromes, genetic testing is available and can potentially quantify an individual's risk for developing specific types of cancer and indicate the need for increased surveillance or preventive measures. Several retrospective studies have reported that patient concern about genetic discrimination is a deterrent to genetic testing for cancer predisposition. In this study, we set out to characterize pre-counseling knowledge and opinions about genetic discrimination amongst cancer genetic counseling patients via a self-administered, confidential survey. The survey gathered demographic information, measured beliefs and behaviors on a Likert-type rating scale and knowledge about protective legislation in a true-false-dont know format. Of 62 consecutive new patients at the City of Hope Cancer Screening & Prevention Program, 46 completed and returned the survey prior to the beginning of the cancer risk counseling session. The majority reported that they were not worried or neutral about genetic discrimination with 20% reporting fear of genetic discrimination. Participants who had never had cancer were significantly more worried about genetic discrimination than participants who had been affected with cancer (p -value = 0.018). The most common answer to the knowledge questions was Dont Know (53%), followed by correct answers (36%), and incorrect information was the least common answer at 11%. Additionally, in our cohort, 41% reported never having heard of the possibility of genetic discrimination before taking the survey. The responses to our study and previous reports indicate that patient, population, and professional education is needed regarding genetic discrimination and that the majority of patients presenting for cancer risk counseling are not worried about genetic discrimination. To determine if fear of genetic discrimination prevents at-risk individuals from pursuing genetic cancer risk assessment and genetic testing, studies of that population are needed.

A study of parental disclosure of genetic test results to young adults, adolescents and children. *A.R. Bradbury^{1,2}, C.K. Daugherty^{1,2}, S. Auh¹, F.J. Hlubocky¹, S. Cummings¹, M. White¹, O.I. Olopade¹.* 1) Hematology/Oncology, University of Chicago Hospitals, Chicago, IL; 2) MacLean Center for Medical Ethics, University of Chicago Hospitals, Chicago, IL.

Genetic testing of minors for adult-onset diseases has generally been discouraged. However, controversy regarding testing continues to exist. One study reported 53% of parents with a BRCA1/2 mutation discussed test results with their minor children. Thus, despite a restriction on genetic testing in individuals under the age of 18, many teenagers and children are being informed of their genetic risk. The purpose of this study is to evaluate the rate of parental disclosure of BRCA1/2 results to adult and minor children, under the age of 25 years old, within our Cancer Risk Clinic. To date, 23 BRCA1/2 carriers having completed an open-ended telephone survey, with 83% of participants women; 91% Caucasian. Median age of probands: 43 years old. Subjects had a total of 49 children; 63% female. The median age of the children at time of testing was 11 years old (25% were 18-25; 34% were 10-17; 41% less than 10 years old). Eight (35%) parents reported disclosure to at least one child; 15 (65%) had not disclosed. In those with children less than 18, 2/18(11%) discussed test results with their children. Of those who did not disclose, when asked when they would share test results with their children, 35% reported plans to disclose before or at the age of 18. Overall, 42% of parents either have disclosed, or plan to disclose, genetic test results to their children prior to the age of 18. When asked if genetic testing of minors should be permitted, 70% responded no, 22% were in favor and 9% were undecided. A significant number of individuals screened within a cancer risk clinic for BRCA mutations either have communicated, or plan to communicate, their genetic test results to their children prior to the recommended age of genetic testing. Despite this, most at risk individuals do not agree with direct testing of children. Further research is needed to describe the communication process among parents and children at risk of BRCA mutations and the impact of these communications on children and family dynamics.

Impacts of whole genome analysis on health management. *K.T. Buck, T. Kreiner.* Corporate Affairs, Affymetrix, Santa Clara, CA.

Genetic testing and the debates surrounding its use have, thus far, been focused primarily on the use of tests for rare and highly-penetrant single-gene disorders. However, as we understand the relative contributions of human variation to different diseases and traits, and interventions are increasingly targeted towards individuals, it becomes possible to imagine screening entire populations for genetic information. Concurrently, the development of whole-genome, high-throughput, and affordable genetic analysis tools make the future possibility of widespread pre-symptomatic population screening both possible and potentially cost-effective.

When considering population screening, it is useful to examine the 50 existing state-run newborn screening programs which, between them, screen the 4 million infants born every year in the US. Newborn screening programs provide a potential infrastructure for future population screening as well as a template for alternative programs that might seek to screen individuals at various points in their life. Those involved with these screening programs have already discussed a number of the issues surrounding genetic testing such as privacy and confidentiality, informed consent, storage and access to biospecimens, diversity in testing requirements, and appropriate clinical follow-up. As the breadth of genetic tests continues to expand, the critical importance of health professional expertise in both the science and the social issues surrounding the use of genetics in medicine also becomes increasingly clear. This is a huge challenge calling for systemic changes in our educational infrastructures as well as increased discussion and debate among scientists, physicians, the media and the public.

In order to capitalize on the promise of genetics and move medicine from crisis-driven intervention to predictive medicine, it will be critical that we take what lessons we can from existing genetic testing and public health screening programs. This presentation will examine these opportunities from the perspective of a technology innovator that has enabled whole genome analysis.

Genetic information: special or not? Responses from focus groups with Health Plan members. *B. Diergaarde¹, E. Ludman², J. Culver¹, D. Bowen¹.* 1) Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Group Health Cooperative, Seattle, WA.

The use of genetic information will become more and more common in medicine. Some experts have argued that genetic information is qualitatively different from other medical information and, therefore, raises unique social issues. This view, called genetic exceptionalism, has importantly influenced policy efforts in the last few years, e.g., special laws to protect against abuse of genetic information. Other experts have argued that genetic information is like other medical information and that treating it differently may actually result in unintended disparities. Little is known about how the lay public views genetic information. To identify opinions about ELSI issues regarding genetic and other medical information among the general population, we conducted 10 focus groups. Participants were women and men between ages 18 and 74, living within 30 miles of Seattle and members of the Group Health Cooperative. A structured discussion guide was used to ensure coverage of all predetermined topics. Sessions lasted ~2 hours; were audio taped and transcribed. Participants recognized that currently their medical information is used and stored at many different locations. They did not view genetic information as being different from other medical information; reported that all sensitive medical information should be treated better; and were particularly concerned about the potential of discrimination and stigmatization for all types of medical information. Personal choice (i.e., right to choose whether to know and to choose and control who else knows what) was reported to be of key importance with the exception of when it could harm others. To conclude, people don't think genetic information is unique and have some understanding of the issues related to sharing medical information. Consensus exists around several key issues.

Assessing genetic services along the Texas-Mexico Border: Surveys of genetics providers. *V.B. Enciso¹, J. Martinez², M.A. Aguilar¹, J. Benkendorf³, J. Cooksey³, S.K. Shapira¹, A. Furino².* 1) Pediatric Genetics, Univ Texas Health Sci Ctr, San Antonio, TX; 2) Center for Health Workforce Studies, Univ Texas Health Sci Ctr, San Antonio, TX; 3) Univ of Maryland School of Medicine, Baltimore, MD.

PURPOSE: This assessment of clinical genetic services in the Texas-Mexico border area is linked to the national study of genetic services and the health workforce (UMB). It focuses on determining how genetics services are provided in Texas, especially to the rural border communities, where distance, culture and language barriers present unique challenges. **METHODS:** Prospective surveys from 2000 and 2001 by the former Texas Genetics Network (TEXGENE) were used to evaluate patterns and characteristics of referrals from border areas to genetic centers, and distances traveled for services. To document changes since TEXGENE data were collected, informal surveys of current genetic centers in Texas were done to update present practices, outreach clinics, and ability to provide services to the border communities. **RESULTS:** Analysis of over 12,000 patient visits from 2000/2001 indicates how genetic services were provided to the border area. Patients who receive services in urban centers may travel 150-350 miles to a genetics center. Outreach clinics located in the border cities of Brownsville, Harlingen, McAllen, Laredo and El Paso decrease patients' travel time and expense to access services. In 2004, surveys of genetics providers indicate that the clinic in Laredo has closed, new outreach clinics started in El Paso, McAllen and Harlingen, and an MD geneticist established a practice in McAllen. Private practice and laboratory-run genetic services increased statewide, but they have largely not benefited the border communities directly. **CONCLUSIONS:** Over the past 5 years there has been expansion of genetic services in urban areas around Texas, but there is little evidence of growth in provision of genetic services to border residents. Genetic providers report that major difficulties are the cost of setting up outreach clinics directly in border cities, and inadequate reimbursement for time spent providing clinical services.

REGULATORY APPROACHES TO REPRODUCTIVE GENETIC TESTING. *R.M. Isasi, B.M. Knoppers.* Centre de recherche en droit public Universite de Montreal, Montral, Quebec, Canada.

This presentation analyzes the ethical and legal aspects of reproductive genetic testing in 11 countries, (Australia, Austria, Canada, France, Germany, India, Israel, Japan, Netherlands, Switzerland and United Kingdom). The legal status of reproductive genetic testing in these countries is difficult to generalize due to the different regulatory systems adopted. These approaches reflect the legal traditions and cultural and socio-religious beliefs which inform and shape public policy on assisted reproductive technologies and genetic testing. Generally, countries favour either a public ordering legislative, top-down model or a private ordering that is a non-legislative and bottom-up model. Even limiting our analysis then to a number of countries that span the range from restrictive to pragmatic approaches, there is remarkable symmetry in both the (I) substantive requirements (i.e. gravity, health indications generally) and (II) procedural safeguards (i.e. informed consent, counselling, confidentiality, civil status, oversight and accreditation). Indeed, irrespective of whether a country adopts a prohibitive or a permissive approach through legislation or self-regulation or a mix of both, the ultimate decision is and should continue to be - a medical one. Nowhere, is this more evident than in the substantive requirements. We will demonstrate how these approaches constitute a useful template for policymaking in this controversial area.

Defining DNA Diagnostic Screening Tests Appropriate for Standard-of-Care. *R.V. Lebo¹, H.E. Wyandt², A. Milunsky².* 1) Dept Pathology, Akron Children's Hospital, OH; 2) Center for Human Genetics, Boston Univ Sch Med, MA.

State-of-the-art clinical testing beyond cystic fibrosis screening for pregnant Caucasian women can be optimized by developing a prioritized list of the most common genetic diseases with their most common mutations that would identify the largest proportion of affected patients among those tested. When developing a microbead, mass spec, or microchip based test with many sites tested per sample, an investigator would simply select the optimal number of sites to be tested, and then proceed down the age-specific list to select the optimal number of highest priority mutations that deliver the most comprehensive test with the highest rate of abnormal pickup that optimally utilizes the available testing format. List items would be prioritized according to (1) frequency of affected patients, (2) frequencies of the most common mutations to be tested, (3) disease severity that helps define the most appropriate age-specific screening category, (4) the targeted test population and whether each tested disease is worldwide or reported in specific ethnic groups, and (5) locations characterizing gene and chromosome aneuploidy. Three disease lists developed according to these principles will be presented for screening fetuses, pregnant couples, and older adults. This approach would provide a more widespread, reasoned testing strategy than individual laboratory offerings sometimes focused on test patents, cost and percentage profit. Updated public lists developed according these principles would provide professionals, laboratories, oversight committees, insurance companies, and government officials an objective means to optimize health care test costs and address ethical issues. Fetal screening could include the common diseases with high de novo mutation rates like Rett syndrome as well as testing aneuploidy for 35 disease genes selected to reveal 98% of chromosome abnormalities in viable newborns. Ethical testing issues would be reviewed according to age-of-onset, disease severity, and counseling issues for each tested age category.

Institutional challenges for expanding genetic health care services: A case study of cancer genetics in Ontario.

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Developments in genetic science are expanding the role of genetics in health care. Recently, cancer care has been undergoing this expansion with the availability of predictive testing services for familial cancers. This trend will likely continue as new research elucidates relationships between genes and a range of common multi-factorial conditions such as Alzheimer's and cardiovascular disease. We report on a study exploring the institutional challenges of moving new genetic technologies into clinical service, using the implementation of BRCA1/2 and HNPCC testing in Ontario as a case study. We conducted (approx.) 50 qualitative interviews with medical and laboratory geneticists, oncologists, genetic counsellors, nurses, and government policy-makers. The participants responded to open-ended questions about the development of the service, the roles of different professionals and decision-making processes. Documentary sources, such as government publications and committee minutes, were also analyzed. Qualitative analysis software (NVivo) assisted the identification of emerging themes and concepts for analysis. Several institutional challenges face the expansion of genomic medicine. In particular, there are clear tensions between professional groups who have distinct, and sometimes contradictory, views of their 'genetic territory', who owns and controls cancer genetics, and who should make decisions about what constitutes appropriate service provision. Geneticists interpret cancer genetics as a genetic service, whereas oncologists understand a genetic test as only one aspect of comprehensive cancer care. These differing views reflect different models of holistic care and different ethical and procedural norms that are not simply reducible to battles over 'turf'. Such tensions create substantial challenges for the translation of research into new genetic services by delaying service provision, producing fragmented care and increasing the costs of implementation. These institutional factors create ethical, economic and practice problems for both policymakers and clinicians.

Risks and benefits in gene transfer research (GTR) involving participants diagnosed with glioblastoma multiforme (GM): an interpersonal approach. *G. Nycum*. Faculty of Law, McGill University, Montreal, CAN.

The Declaration of Helsinki states: In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society. It also states: Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject and that in the evaluation of research protocols [s]pecial attention is required [...] for those who will not benefit personally from the research.

These statements sanction research with an important objective even where the objective is not a benefit to participants. An example of this type of research is GTR involving participants diagnosed with GM. Since direct medical benefits to these participants are unknown, the risk/benefit analysis involves direct non-medical benefits and indirect benefits to society and future patients.

In the US, a web of institutional bodies review GTR protocols. In the case of non-medical and indirect benefits, the risk/benefit analysis should be difficult at the institutional level because every participant will have a personal and unquantifiable encounter with non-medical and indirect benefits.

Institutional bodies must ensure that researchers and institutions are knowledgeable about the potential for coercion and therapeutic misconception on the part of terminally ill participants who will not benefit personally from research; as well as the importance of participant autonomy, especially for those at the end of life. Because direct benefits may be unquantifiable, in the review of GTR, institutional bodies should put forward the importance of interpersonal communication between researchers and participants. Part of the institutional review process should be the design of education and tools for researchers for communicating with potential participants. This would ensure that the substantive value of the protection of human subjects is not diluted by institutionalized procedures such as the acquirement of informed consent.

Genetic origins of homosexuality: Public beliefs and social implications. *E.M. Petty¹, C.A. Pfeffer², M.B. Feldbaum², T.E. Jayaratne², J.P. Sheldon³.* 1) Med Sch, Univ Mich, Ann Arbor, MI; 2) IRWG, Univ Mich, Ann Arbor, MI; 3) Behav Sci, Univ Mich-Dearborn, Dearborn, MI.

Diverse theories on the origins of homosexuality exist. Some suggest a genetic basis. Given the complexities and controversies surrounding sexual orientation, we sought to explore public beliefs about genetic contributions to homosexuality. We conducted open-ended IRB-approved phone interviews with 86 Black and White adults from across America to learn their beliefs on the origins of homosexuality, what shaped their beliefs, and their views on implications. Responses were analyzed for thematic content. Half felt genes played no causative role, noting factors from choice and upbringing to Satan. Eight felt homosexuality was totally genetic, 25 felt it was partly genetic, and 5 felt genes played a role only in some gays. A third cited factors in 2 or more areas: genetic/biological, environmental, choice, and other. Twenty-six confused sexual orientation with sex: Youve got so many X and Y chromosomes...you get more of one chromosome when its a homosexuality person. Others confused it with gender roles. Beliefs were shaped by: gay friends/acquaintances, media, gay family members, religious teachings, non-gay family/friends, science, and personal experiences. Family trees influenced half (e.g. it can't be genetic if only one family member is gay). On how finding genetic factors may help people, positive responses included: increase heterosexuals tolerance, help gays self-acceptance, and show homosexuality isnt a choice. Responses on developing cures, gene tests, and prenatal tests implied that eradicating homosexuality would help. On how genetics could be harmful responses included: increase hate/discrimination, change/eliminate genes, and increase family blame. Some noted harm could result by legitimatizing homosexuality. We found that limited genetic understanding led to simple reasoning concerning heritability of a complex trait and that genetics was used to support diverse opinions, including negative eugenic agendas. Thus, caution is needed as we seek genetic mechanisms underlying complex human traits; especially those that may impact oppressed minority populations.

Predictive genetic tests for neuropsychiatric diseases. *M.DC. Rietschel^{1,2}, F. Illes^{1,2}*. 1) Genet Epidemiol Psychiat, ZI, Mannheim, Germany; 2) Dep. Psychiat, Univ. Bonn, Germany.

Introduction

Neuropsychiatric diseases are caused by an interaction between various susceptibility genes and environmental factors. These genes are currently being identified and predictive genetic testing may soon become feasible. The contribution of each gene is small, and predictive testing based on single genes will not allow prediction of onset and progression in individual cases. The aim of our study is firstly to assess public attitudes to genetic testing and secondly, to measure the level of understanding and ability to interpret risk amongst the general population.

Methods

We chose Alzheimerss disease (AD) as an example of neuropsychiatric disease. It is well known, everyone has a substantial risk of becoming affected, and a risk gene (APOE4) has already been identified. A representative sample of the German general population (n = 2000), 83 relatives of AD patients and 43 physicians were interviewed regarding their attitudes towards genetic tests for AD, and their perception and interpretation of risk. The study is ongoing and to assess the influence of genetic counseling, 200 people are questioned before and 4 weeks after they have been provided with detailed relevant information.sp; Results

Our results show that the majority (85%) of the representative sample is in favour of predictive genetic testing for AD. 43% of the general population, 50% of the relatives and 30% of the physicians expressed an interest in being tested for APOE4. 25% of the general population, 10% of relatives, and 2% of physicians were in favour of prenatal testing. The majority had difficulty interpreting risk information. Only 34% understood the meaning of risk estimates given as percentages. Preliminary results suggest that genetic counselling significantly improves this understanding.

Women's perspectives on genetics: understanding, expectations and values. *B. Wilson¹, B. Potter¹, Y. Chorny¹, I. Boland¹, I. Graham², M. Walker², D. Coyle².* 1) University of Ottawa, Ottawa, Ontario, Canada; 2) Ottawa Health Research Institute, Ottawa, Ontario, Canada.

Purpose: This project is designed to examine the relationship between what people actually understand about genetics, their personal values on the acceptable use of genetic technologies, and their expectations of what it is appropriate for a publicly funded health service to provide. These issues are being explored in a sample of individuals likely to have considered some of them in a personal way, i.e. women who have been offered routine screening tests in past pregnancies. **Methods:** This is the first part of a multi-method study. Women who delivered a healthy child at the Ottawa Hospital between 1994 and 2004 are recruited using a purposive sampling strategy. Data collection is by semi-structured interviews. Thematic analysis is conducted as the data are collected, allowing for an evolving range of issues to be explored. A modified grounded theory approach is used for the analysis of transcripts. Approximately 72 women will be interviewed by fall 2004. **Results:** Key themes emerging so far are: (1) influences on the perceived personal value of testing (e.g. personal notions of risk, understanding of the difference between predictive and diagnostic tests, the value of information as an end in itself, the perceived value of medical technologies); (2) personal decision-making processes (e.g. the role of social networks and shared experiences, active and passive decision-making styles); (3) the importance of freedom of individual choice (e.g. others should be allowed to make their own choices irrespective of the views of the interviewee, the perceived importance of self-awareness); and (4) issues related to the health care system (e.g. fair access to services, willingness to pay, potential targeting of genetics services, the concept of necessity, differences between prevention and treatment goals). **Anticipated value:** This study will provide insight for policy decision makers and providers who wish meaningful feedback from citizens and service users. It will also provide evidence on the utility of methods for public engagement in health care decision making.

Molecular analysis of 32 common CFTR mutations in Chilean patients with cystic fibrosis. *G.M. Repetto¹, A. Puga¹, M.L. Boza², M.A. Perez³, R. Kogan³, O. Fielbaum⁴, C. Pierry⁵, M. Linares⁶, M. Mendez⁷, H. Poggi⁸, R.A. Pardo⁸, E. Romeo⁸, A.C. Foradori⁸, P. Astudillo⁹.* 1) Dept Genetics, Univ del Desarrollo-Clinica Alemana; 2) Hospital San Borja Arriaran; 3) H. Exequiel Gonzalez Cortes; 4) H. Luis Calvo Mackenna; 5) H. San Juan de Dios; 6) H. Padre Hurtado; 7) H. Sotero del Rio; 8) P. Universidad Catolica de Chile; 9) Ministerio de Salud, Santiago, Chile.

A National Cystic Fibrosis (CF) Program was initiated in Chile in 2000 to improve access to diagnosis and therapy for known patients in the country. Two hundred and seventy patients have been registered, accounting for more than 90% of known patients. With a mean survival age of 12 years, this would imply a disease incidence of about 1 in 15,000, but the actual figure is unknown. We report initial data on molecular analysis on patients in the program and an evaluation of the frequency of F508 carriers in healthy population to estimate the disease frequency. DNA samples from 60 unrelated patients were analyzed for the presence of 32 "common" mutations by PCR-OLA using the commercial assay Cystic Fibrosis v3.0 (Celera Diagnostics USA). The detection rate was 38.5% and 6 mutations were found: F508 in 27% of alleles, R334W in 5%, G542X in 3.3%, 3849+10kb CT in 1.6%, W1282X and 1078delT in 0.8% each. The relatively high frequency of R334W and G542X is consistent with the Spanish contribution to Chilean population admixture. The presence of F508 was evaluated by allele specific PCR in 300 healthy individuals representative of the mixed population of Santiago. The mutation was found in 2 alleles. Given these data, the carrier rate for any mutation was estimated to be around 1 in 40 and the disease frequency, 1 in 6400. The results are similar to other Latin American countries and emphasize the need to improve clinical and molecular diagnosis of CF in the region.

Cost Comparison of Genetic and Clinical Screening in Families with Hereditary Hemorrhagic Telangiectasia.

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Background: Endoglin (ENG) and ALK-1 mutations cause Hereditary Hemorrhagic Telangiectasia (HHT), an autosomal dominant disorder leading to vascular dysplasia in the form of mucocutaneous telangiectasia and visceral arteriovenous malformations (AVMs). **Aims:** To compare costs of two alternative strategies for management: screening HHT families by a molecular diagnostic test followed by clinical screening only for individuals who carry the family's mutation versus conventional clinical screening. **Methods:** Direct health care costs associated with clinical and genetic screening for a hypothetical HHT family were evaluated using a decision analytic model. The family consists of one index case (an individual with a clinical diagnosis of HHT) and 13 of their relatives. The clinical screening protocol was determined from the medical literature and expert opinion from the Canadian HHT Center in Toronto. Unit costs for clinical screening were in Canadian dollars and were obtained from the 2002 Ontario Health Insurance Schedule of Benefits. Genetic screening costs were estimated for Quantitative Multiplex PCR and sequencing of Endoglin (ENG) and ALK-1 genes, as performed at HHT Solutions, Toronto. **Results:** Based on a weighted average of all family members screened, it was determined that the genetic/targeted clinical screening strategy costs \$3544 per individual versus \$4933 for the clinical screening strategy. Thus performing genetic screening strategy would save \$1389 per family member that totals \$18,057 saved per family. Sensitivity analyses revealed that the genetic screening strategy was cost saving over all plausible ranges of input variables for all hypothetical families tested.

Workplace screening for hemochromatosis does not cause psychological morbidity in participants and reveals an increased rate of fatigue in C282Y homozygotes. *M. Delatycki^{1,2}, A.E. Nisselle¹, V. Collins¹, S. Metcalfe^{1,2}, M.A. Aitken^{1,2}, D. duSart¹, J. Halliday^{1,2}, I. Macciocca¹, A. Gason^{1,2}, R. Williamson^{1,2}, K.J. Allen^{1,2}.* 1) Murdoch Childrens Research Institute, Victoria, Australia; 2) Department of Paediatrics, University of Melbourne, Victoria, Australia.

Introduction- There is debate as to whether community genetic screening for hemochromatosis should be implemented, due to issues including disease penetrance, health economic outcomes and concerns about community acceptance. Hemochromatosis is a common, preventable iron overload disease, due in over 90% of cases to C282Y homozygosity in the *HFE* gene.

Methods- C282Y screening was undertaken in the workplace. Questionnaires were administered following group education and to a subcohort following result disclosure. Anxiety was measured by the STAI.

Results- 11 042 people had screening. Analysis is from the first 10 472 participants. Of those eligible, 9.3% (range 3-86%) attended information and screening sessions, of whom 93.3% chose to be tested. 48 C282Y (1:218) homozygotes and 1195 heterozygotes (1:8.8) were identified. 22/34 (65%) C282Y homozygotes had abnormal iron indices. One C282Y homozygote fulfilled the criteria for liver biopsy (ferritin 4036g/L) and had precirrhotic fibrosis. C282Y homozygotes had a significantly higher rate of fatigue (CC- 57.6%; CY- 59.2%; YY- 81.8%. $\chi^2 = 8.94$, $p=0.01$). There was no significant difference seen between the groups for abdominal pain, arthritis or liver disease. Pre and post test anxiety was not significantly different in C282Y homozygotes compared to heterozygotes or wildtype homozygotes. There was a non-significant decrease in anxiety with the diagnosis of C282Y homozygosity or heterozygosity (CC pre 34.3, post 35.3; CY pre 35.4, post 30.0; YY pre 35.2, post 32.1).

Conclusion- Widespread screening for hemochromatosis is readily accepted in a workplace setting and C282Y homozygosity does not result in increased anxiety. There is an increased rate of fatigue among C282Y homozygotes.

A randomized trial on acceptability of DNA versus blood marker testing to detect hemochromatosis in primary care settings: The HEIRS study. *R.T. Anderson¹, N. Press², D. Tucker³, L. Wenzel⁴, B. Snively¹, M. Hall¹, S. Ellis¹, A. Walker⁴, E. Thomson⁵, O. Lewis-Jack⁶, R.T. Acton³.* 1) Wake Forest University, Winston-Salem, NC; 2) Kaiser Permanente Center for Health Research, Portland, OR; 3) University of Alabama at Birmingham (UAB), AL; 4) University of California, Irvine (UCI), CA; 5) National Human Genome Research Institute, Bethesda, MD; 6) Howard University, Washington, DC.

Genetic screening may enable timely and effective detection and treatment of hemochromatosis (HH) in primary care settings. However, little information is available about the acceptability of DNA testing vs. conventional blood marker tests of iron stores. Within the HEIRS Study, a multi-site primary-care-based screening study for HH, a separate study was conducted to assess patients willingness to be tested for HH by type of test among black and white patients. Participants were randomized to receive brief information on HH DNA or blood marker testing; study outcomes included willingness to be tested and attitudes toward HH testing. Results: A total of 2,186 participants were recruited from waiting rooms of primary care practices prior to contact by HEIRS staff, of these 2,165 met the final study criteria. Adjusting for age, Field Center, gender, educational attainment and global health rating, there were no differences in acceptability by DNA (55.8%) versus blood marker (58.6%), $p=.078$. Among Field Centers, blacks in Alabama were least likely to accept either form of test (44.7% and 49.6%) than whites, as were participants who were: aged 45-64 years, female, or who rated their health less than very good. Accepting HH testing was associated with the desire to know more about health (81%), and to help family members (71%). Reasons for refusing testing included a need for more information about HH from doctor (44%), privacy concerns (32%) and dislike of blood tests (29%). Conclusion: DNA testing for HH was not less acceptable than blood tests of iron stores in this diverse patient sample. Patient education on nature of test, importance of HH, and privacy protection appear to be essential for achieving high rates of participation of either test.

Detection of muscular dystrophy genotypes via universal condition direct sequencing (UCDS). *R.R. Bennett¹, H.E. Schneider¹, R.C. Nutter², P.S. Lai³, C.E. Barrett¹, V. Lip¹, B.L. Wu¹, B.T. Darras¹, L.M. Kunkel¹.* 1) Genetics, Children's Hosp, Boston, MA; 2) Applied Biosystems, Foster City, CA; 3) Department of Pediatrics, National University of Singapore, Singapore.

Currently, mutations in many of the genes causing the various forms of muscular dystrophy remain undetected in patients because of the unavailability of a reliable and cost effective assay to detect them. One promising strategy requires the amplification of all salient portions of a gene or many genes at one single set of PCR conditions. This is achieved by designing amplicons of uniform large (~1000bp) size. Internal primers are then used for sequencing (Flanigan et al. 2003). A second strategy being employed by Applied Biosystems, Foster City Ca. in its VariantSEQr resequencing system is to use automated primer design tools to seek primers for all salient portions of many genes. Using Amplitaq GoldPCR system, these amplicons will all amplify at a single set of PCR conditions. In addition, by using M13F and M13R tails on the primers, a universal sequencing plate can be utilized in this process. We have developed a high throughput 384 well plate robotic process with dried primers using the Applied Biosystems VariantSEQr resequencing system for genes associated with any form of muscular dystrophy. The new sequencing strategy provides more than an order of magnitude reduction in cost, time to detect mutation and complexity of analysis. Detection of mutations is facilitated rapidly using SeqScape v2.1 software (Applied Biosystems, Foster City Ca.). This software incorporates a consensus sequence based on the NCBI database, complete gene annotation of all NCBI and Celera transcripts for the gene of interest and a tab process for tabing through all variations between patients and consensus. It also features rapid identification of known polymorphisms versus unknown variants as well as insertions and deletions. As of June 2004, we have tested this process called UCDS (Universal Condition Direct Sequencing) for the DMD, CAV3, TRIM32, CAPN3, FKRP and FCMD genes using control DNA and two DMD patients. More genes and more patients will be studied over the summer.

Immunodetection of myotubularin for expression studies and for diagnosis of myotubular myopathy. A. Buj-Bello, J. Laporte, G. Duval, J.L. Mandel. Molecular Pathology, IGBMC, CNRS/INSERM/ULP/College de France, ILLKIRCH, France.

X-linked myotubular myopathy (XLMTM) is a severe congenital myopathy characterized by generalized hypotonia and respiratory insufficiency at birth. The gene responsible for the disease, MTM1, encodes a phosphoinositide phosphatase named myotubularin with an essential function in skeletal muscle that is conserved in mice, as *Mtm1* deficient (KO) mice develop a progressive centronuclear myopathy during postnatal life leading to death. MTM1 belongs to a large disease-associated gene family with 14 members in humans, which includes MTMR2 and MTMR13, mutated in Charcot-Marie-Tooth demyelinating neuropathies type 4B1 and 4B2, respectively. Direct sequencing of the 15 MTM1 exons and flanking intronic sequences generally establishes the molecular diagnosis. More than 300 mutations have been identified in approximately 85% of patients with an XLMTM-like phenotype. Mutations in some patients may be located in the promoter or in other intronic regions of the gene, which are not routinely analyzed. We have established a novel protocol by immunodetection of myotubularin that can be used as an alternative approach for diagnosis of XLMTM. For this, we have generated a novel polyclonal antibody against myotubularin that can detect the endogenous protein by direct western blotting. We prepared protein homogenates from lymphoblast, fibroblast and myoblast cell lines and found abnormal levels of myotubularin in most analyzed XLMTM cell lines, with either a decrease or absence of the protein. More importantly, we are also able to detect myotubularin in lymphocytes isolated from peripheral blood of normal individuals, which should avoid transforming cells to apply this procedure. This technique may thus be useful for molecular diagnosis, especially in cases in which MTM1 mutations have not been found and to analyse the effect of variants of uncertain pathogenicity on expression of the protein. In addition, we have analyzed myotubularin expression during mouse development by immunoblotting and observed a postnatal accumulation of myotubularin in skeletal muscle, that correlates well with the postnatal onset of muscle pathology in *Mtm1* KO mice.

Cost-effectiveness of a schools-based Tay-Sachs disease (TSD) and cystic fibrosis (CF) genetic carrier screening

program. *L. Burnett*¹, *E. Warren*², *R. Anderson*², *A. Proos*¹, *K. Barlow-Stewart*³, *J. Hall*². 1) Pacific Laboratory Medicine Services (PaLMS) and Kolling Institute of Medical Research, Royal North Shore Hospital, St Leonards, NSW, Australia; 2) Centre for Health Economics Research and Evaluation (CHERE), University of Technology Sydney, Sydney, NSW, Australia; 3) Centre for Genetic Education, NSW Genetics Service, Royal North Shore Hospital, St Leonards, NSW, Australia.

Aims: To explore cost-effectiveness (CE) of a school-based two-disease genetic carrier screening program, compared with no school-based screening and various other scenarios.

Method: A decision analytic model was developed to estimate the incremental CE of a school-based TSD and CF genetic carrier screening program, relative to no screening program. The cost of the program was built up using a micro-costing approach, and effectiveness measured as the number of genetic carriers detected. Data relating to ethnicity, test-accepting behaviour and result notification schemes were sourced from a reference program in Sydney, Australia. The impact on CE of underlying assumptions in both screening and no screening alternatives has been explored in sensitivity analyses.

Results: The estimated annual cost of the reference school screening program for TSD and CF is A\$137 per student screened. Relative to no screening program, the incremental CE of the reference program is ~A\$6,000 per additional carrier detected, implying a cost per TSD or CF child avoided of ~A\$530,000. More efficient strategies for detecting genetic carriers have been identified, including restricting access to testing only to those at increased *a priori* ethnic risk; this reduces the cost per additional carrier detected to ~A\$5,000. Sensitivity analysis shows that the CE ratio is most sensitive to changes in genetic test accuracy, cost of laboratory assays, and the relative weighting CF and TSD genetic carriers. The model allows estimation of the cost of any future equally efficient non-schools-based public awareness campaign for genetic carrier screening.

Controversies in determining appropriate uses of genetic information in the diagnosis, treatment and prevention of Autosomal Dominant Polycystic Kidney Disease. *S. Cox, S. Yew, B. Askwith.* Centre for Applied Ethics, University of British Columbia, Vancouver, BC, Canada.

Aims: This qualitative study investigates patient, family and health care provider perspectives on the uses of genetic information in the diagnosis, treatment and prevention of autosomal dominant polycystic kidney disease (ADPKD). The aim is to inform appropriate clinical practice, patient education, health services and policy. **Background:** ADPKD is a common hereditary disorder that causes fluid-filled cysts on the kidneys and progressive loss of renal function. Diagnosis is typically made using ultrasound although the absence of renal cysts is not definitive for persons < 30 years. Genetic testing of the PKD1 and PKD2 genes by linkage or mutation analysis is available but uptake for presymptomatic and prenatal testing in Canada is low. **Methods:** In-depth interviews with 30 PKD patients and family members, and 15 health care providers were conducted in Canada in 2003-2004. Thematic analysis of the data focuses on awareness of the availability of genetic testing, perceived clinical utility, and associated benefits and harms. **Findings:** Awareness and perceived utility of genetic testing varies widely amongst persons at risk for or diagnosed with PKD, family members and health care providers. Three case studies featured in this presentation exemplify social, ethical and clinical challenges in using genetic testing for: 1) presymptomatic diagnosis, 2) identification of potential living related kidney donors, and 3) targeted surveillance for ADPKD patients at high risk of associated complications such as cerebral aneurysm. **Conclusion:** Clarification of social and ethical as well as clinical controversies surrounding the utility of genetic testing for ADPKD will lead to the development of criteria that assist health care providers, policymakers, nonprofit associations, patients and families in defining appropriate uses of genetic information. In the next phase of research, the controversies identified here will be used to stimulate dialogue in focus group discussions with a range of ADPKD stakeholders.

Frequent finding of duplications in the DMD gene in patients with Duchenne / Becker muscular dystrophy identified by MLPA. *R. Fahsold, K. Hager, C. Ehlers, H. Monir.* Institute Med. Genetik, Dresden, Germany.

The dystrophinopathies - the severe type Duchenne (DMD) and the milder type Becker (BMD) are the most common inherited disorders of muscle with a prevalence of 1:3500 live male births. Both BMD and DMD are X-linked diseases resulting from a defect in the huge gene DMD (~2,4 Mb of genomic sequence), that encodes the protein dystrophin. In most studies, deletions of one or more exons are found in approximately 65 % of patients; duplications account for 6 % of mutations, and point mutations account for the remainder. The detection of copy-number changes in the DMD gene, especially the verification of duplications in effected males, was only possible with complex, specific and labor intensive quantitative techniques (e.g. Southern blotting and real-time PCR). But now there is a simple and effective method to establish the copy number of specific sequences: Multiplex Ligation-dependent Probe Amplification (MLPA). With MLPA, it is possible to quantify all of the 79 DMD-Exons simultaneous in only two multiplex PCR reactions. Our experiences and results with the use of MLPA shows, that the rate of duplications in effected males seems to be higher than expected in previous studies. We will present our cases, for example a novel duplication in the 3-region of the DMD gene.

Non-Profit Reference Laboratories to Implement Genetic Tests for Complex Orphan Diseases. *B. Gallie*^{1,2,3,4}, *K. Vandezande*^{2,3}, *M. Letarte*^{3,4}. 1) Ontario Cancer Institute, Toronto ON; 2) Retinoblastoma Solutions, Toronto, ON; 3) HHT Solutions, Toronto, ON; 4) The Hospital for Sick Children, Toronto, ON, Canada.

We document ongoing projects to establish self-sustaining reference labs for two orphan genetic disorders: retinoblastoma (RB) and hereditary hemorrhagic telangiectasia (HHT). Both diseases result from mutations in large genes and each affected family has its own unique mutation. We formed non-profit corporations to achieve the highest quality testing at affordable cost, developed molecular assays for each class of potential mutation, and optimized for maximum test sensitivity and minimum turnaround time. For both RB and HHT, we are achieving the highest test sensitivity yet documented. Despite the inherent complexity of mutation detection for these diseases, clinical impact studies suggest that optimized molecular tests can achieve lower healthcare cost and higher quality of life. Standard care without molecular knowledge requires frequent, expensive and invasive clinical screening. Health outcomes improve when molecular tests identify carriers of a familial mutation for early intervention. Health costs are reduced when individuals at risk are shown not to carry a mutation and no longer need clinical surveillance. For these two complex disorders, optimized molecular diagnosis improves the quality of life and decreased health costs, indicating that the application of genome knowledge to healthcare does not necessarily increase costs. Public clinical diagnostic labs are ill-funded for technical and economic optimization of tests, while private industry sees investment risks in molecular tests for rare and difficult disease genes. We propose that self-sustaining labs with disease-specific focus should receive national and international support and become "global" reference labs for a specific number of orphan diseases. We find that non-profit charitable form offers distinct advantages for reference labs over alternative organizational structures.

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Quantitative PCR for the rapid detection of deletions in Hunter syndrome. *J.R. Jones, H. Risheg, T.C. Wood, M.J. Friez.* Greenwood Genetic Center, Greenwood, SC.

Hunter syndrome or mucopolysaccharidosis type II (MPS II) is an X-linked recessive lysosomal storage disease resulting from a deficiency of the enzyme iduronate-2-sulfatase (IDS). Features include mental retardation, short stature, coarse facies, skeletal deformities, stiff joints, and cardiovascular disorders. Molecular analysis has identified many different types of alterations in the IDS gene, including small deletions, missense, nonsense, and splice site mutations. In addition, a variety of techniques have identified large deletions in a small fraction of cases. Unfortunately, when large deletions are identified in affected males, it is difficult to determine whether female relatives are carriers of the deletion. In order to assess carrier status in at-risk females, we have developed a rapid method for determining IDS gene dosage using real time PCR. During the validation of this technique, we compared the absolute quantitation method using 3 reference genes to the comparative threshold cycle method (Ct) using only RNaseP as the internal reference. Testing was performed using DNA samples from females with affected male relatives who were suspected of having a deletion based on the failure of PCR to amplify the coding region of IDS. Thus far, 4/4 males have been confirmed to have a deletion involving the IDS gene, and 2/5 females have been found to carry a deletion. Interestingly, one of the 2 carriers is an affected female with highly skewed X inactivation. The Ct method gave consistent results while the absolute quantitation method showed some variation and discrepancies in results possibly due to deviations within the standard curves. Our results indicate that the Ct method of real time PCR is a rapid, reliable approach for the identification of IDS gene deletions in affected individuals and carrier females.

Cystic Fibrosis Carrier Testing: The Collection and Utility of Ethnicity/Race Information on the Laboratory

Requisition Form. *I.M. Lubin^{1,5}, M. Caggana^{2,5}, D.J. Wolff^{3,5}, C. Constantin¹, L. Kalman¹, J.A. Nowak^{4,5}.* 1) Centers for Disease Control and Prevention, Atlanta, GA; 2) New York Dept. Health, Wadsworth Center, Albany, NY; 3) Dept. Path./Lab. Med., Medical University of South Carolina, Charleston, SC; 4) Dept. Path., Advocate Lutheran General Hospital, Park Ridge, IL; 5) Association for Molecular Pathology, Clinical Practice Committee, Bethesda, MD.

DNA-based cystic fibrosis (CF) carrier testing is useful for counseling prospective parents about their risk of having an affected child and other family members at risk. Knowledge of the patient's ethnicity/race is used to calculate residual risk when no mutations are found. This report describes the variation in clinical laboratory requisition forms (n=32, commercial and academic) in requesting ethnicity/race data and implications for calculating residual risk. All requisitions requested ethnicity/race information. The majority of requisitions was designed for ordering multiple tests and the ethnicity/race information requested may be pertinent to tests other than CF. Eight (25%) requisitions requested such information but did not provide specific choices. The remaining laboratories offered 2 to 11 choices including the option to specify a category not listed. ACMG and ACOG CF guidelines provide carrier risk data for five ethnic/racial groups. Of the laboratories offering specific descriptors (n=24), 14 (58%) provide additional choices and 4 (17%) provide fewer. Six requisitions (25%) offered five choices, not necessarily matching those in the guidelines. Carrier frequency data for certain CF mutations are available in the literature and some laboratories use additional data ascertained locally. Risk estimates can therefore be variable based upon what ethnicity/race information is used. There is a need for practice guidelines in the selection, collection, and use of ethnicity/race information to assure appropriate determination of residual risk. Admixture is a related topic requiring consideration. These issues will gain added significance as we expand beyond CF to other carrier and predictive tests that will influence health care decisions.

Attitudes regarding testing adolescents for carrier status. *T. Mulhaupt*^{1,2}, *A. Lovell*^{1,2}, *L. Mills*², *R. Hopkin*^{1,2}. 1) Allied Health Sciences, University of Cincinnati; 2) Cincinnati Children's Hospital Medical Center.

Based on the premise that there are no immediate medical or psychosocial benefits, a position statement published by ASHG/ACMG recommends deferral of testing adolescents for carrier status until adulthood in most cases. To characterize the application of the position statement, we determined factors that genetic service providers considered influential in a decision to test adolescents for carrier status and if their knowledge of the position statement was helpful in considering this issue. We invited genetic counselors, medical geneticists and genetic nurses to participate in an online survey. We measured the factors that influenced the decision by asking subjects to rank the top five of sixteen reasons for testing in their most recent case. The factor that was selected most frequently was judged the most influential. We assessed the use of the position statement by asking subjects to rate the helpfulness of their position statement knowledge. 152/181 subjects (84.0%) tested an adolescent within the last year; 112 provided their reasons for testing. The decision to test was most influenced by adolescent desire (15.3%), adolescents competence/understanding (13.8%) and adolescent sexual activity/pregnancy status (12.0%). Overall, more subjects reported their knowledge of the position statement as unhelpful (22.9%) than those who reported their knowledge as helpful (13.7%). Ensuring that adolescents participate in the decision was a priority for those clinicians who offered testing. Although adolescent desire and competence/understanding are discussed in the position statement as reasons testing may be considered, it does not address the issue of carrier status testing in sexually active or pregnant adolescents or many other reported factors. Also, many subjects were unsatisfied with their knowledge or unfamiliar with the position statement recommendations. These findings suggest: 1) carrier testing is commonly offered to adolescents in the clinical setting, 2) the current position statement fails to address important issues relating to testing adolescents for carrier status, and 3) the position statement is not familiar to many genetic service providers.

A fourteen year technical, ethical and social struggle towards creating a model to prevent -thalassemia in Iran. *H. Najmabadi^{1,2}, A. Ghamari^{1,2}, F. Sahebjam¹, R. Kariminejad¹, T. Khatibi¹, A. Samavat³, E. Mehdipour¹, B. Modell⁴, M.H. Kariminejad¹. 1) Genetic Research Ctr, Kariminejad Najmabadi Genetics, Tehran, Iran; 2) The social welfare and rehabilitation sciences university, Genetic Research Center, Tehran, Iran; 3) Center for Disease Control, Deputy for Non-communicable Disease, Tehran, Iran; 4) RF & UCMS Dept of Primary Care & Population Sciences, and UCL Centre for Health Informatics and Multiprofessional Education (CHIME), Holborn Union Building, Whittington Campus, London, UK.*

For fourteen years Iranian scientists have worked to develop a national thalassemia prevention program. Historically abortion was considered unacceptable in Iran; however, intensive consultations led to the clerical approval of therapeutic abortion of cases with major type of -thalassemia in 1997, and a nation-wide prevention program with screening, counseling and prenatal diagnosis networks has been developed. This paper reports the experience from one of the two national prenatal diagnosis reference laboratories. From 1990 to 2003 we performed a total of 906 prenatal diagnoses from 718 families at risk for thalassemia. Direct and indirect mutation detection methods were applied for all cases. In total, 22 mutations were tested routinely and an additional 30 rare mutations were identified. 208 fetuses were found to be normal, 215 fetuses were major, and 435 fetuses were trait. In 40 cases we only defined one allele. We were unable to provide 8 cases with any diagnosis, corresponding to 0.9%. Our data supports the functionality of Iranian -thalassemia prevention program. The success of this system in Iran as a multiethnic and Islamic-based country would mean that it might be applied as an adaptive system for neighboring and other Islamic countries.

Communicating BRCA1 and BRCA2 test results: Data from a telephone interview with 332 women tested within the German Consortium on Hereditary Breast and Ovarian Cancer. *I. Nippert¹, C. Vogel¹, L. Wingen², D. Gadzicki², B. Schlegelberger².* 1) Women's Health Research, UKM, Muenster, Germany; 2) Institute of Cell and Molecular Pathology, MHH, Hannover, Germany.

Introduction: In order to promote safe and effective testing for BRCA1/2 mutations in clinical practice the German Consortium on Hereditary Breast and Ovarian Cancer (HBOC) has been established. To improve practice, evaluations based upon the views of patients who have undergone testing concerning the impact of the genetic diagnosis and the quality of the services they receive and require are undertaken. **Methods:** 332 women from HBOC families who had obtained their test results at least 6 months earlier were interviewed by telephone. The interview includes open and standardised questions. **Results:** Almost all women (98%) had consulted at least one family member before undergoing testing. 10% reported conflicts with at least one family member about whether or not the test should be taken. The communication process about the test was characterized by selective and preferential information of female family members both before testing and after obtaining the test result. Women with breast cancer showed a greater tendency to inform their children than women without breast cancer. 10% were asked by at least one family member not to tell results. Mutation carriers reported more conflicts with other family members in communicating test results than non-carriers (22% vs. 3%, $p < 0.05$). **Discussion:** Genetic counselling needs to raise awareness that both sexes may inherit the mutation and may benefit from information. Strategies need to be developed and evaluated regarding how to facilitate the dissemination of information within families without potentially overstraining the messenger patient at least when a mutation has been found..

Carrier frequency of familial hyperinsulinism mutations in Ashkenazi Jews. C. Oddoux, L. U, C.M. Clayton, H. Ostrer. Div Human Genetics, New York Univ Sch Medicine, New York, NY.

Familial hyperinsulinism (HI) is characterized by severe, recurrent hypoglycemia associated with an inappropriate elevation of serum insulin. It presents in neonates and infants and carries a substantial risk of death or severe brain damage and mental retardation.

The disorder is heterogeneous and variable and has been found to be caused by both recessive and dominant mutations in several genes including most commonly the SUR1 (ABCC8) and the KIR6.2 genes. Together, these encode the pancreatic beta cell K-ATP channel. Both are found on chromosome 11p15.1. In addition, 30% of HI cases represent a focal form where paternally inherited mutations in either SUR1 or KIR6.2, paired with *somatic* deletion of this imprinted region from the maternal chromosome results in an imbalance in growth factors leading to hypertrophy of pancreatic beta cells that are hemizygous for the paternal mutation and consequently over express insulin.

Estimates of the frequency of HI range from 1:50,000 births in Europe to as much as 1:2,500 births in isolated populations in which there are founder mutations. Among Ashkenazi Jews, two founder mutations in the SUR1 gene have been identified which account for 88% of detected mutations and most likely represent >95% of heritable mutations in light of the contribution of somatic deletion. Using a novel fluorescence assay we analyzed 900 Ashkenazi Jewish individuals to determine the carrier frequency of these two mutations: 3992-9 g->a and F1388. Six carriers of 3992-9 g->a and one carrier of F1388 were detected resulting in an overall carrier frequency of 0.8%. This frequency is comparable to that of Bloom syndrome and Fanconi anemia, which are commonly screened for in this population. Carrier screening for HI in this population provides a valuable means of early diagnosis to guide clinical management of patients which cannot easily be provided by a newborn screening program because of the early onset, high morbidity, and significant contribution of paired heritable and somatic changes in this disease.

A novel approach to ensuring the quality of DNA sequencing output - an international external quality assessment scheme. *S. Patton*¹, *A. Wallace*². 1) European Molecular Genetics Quality Network (EMQN), c/o National Genetics Reference Laboratory, St Mary's Hosp, Manchester, United Kingdom; 2) National Genetics Reference Laboratory, St Mary's Hosp, Manchester, United Kingdom.

Genetic testing is now a routine part of laboratory medicine. Many different technical approaches are used but DNA sequencing is widely regarded as the gold standard for mutation detection. But how accurate is DNA sequencing, and can we be sure we are getting the correct result 100% of the time? External Quality Assessment (EQA), or Proficiency testing, is one approach to independently quantifying the accuracy of the sequencing output from testing laboratories and sequencing facilities. We will present the results of novel EQA scheme for DNA sequencing run by the European Molecular Genetics Quality Network (EMQN) in 2003. The scheme consisted of two parts; a participant questionnaire and a practical assessment designed to assess the SENSITIVITY and ACCURACY of DNA sequencing and review the QUALITY of data provided. Test samples contained a range of different genotypes covering all the main types of sequence change. Results were evaluated for genotype and output quality. Sixty-one laboratories from twenty-one countries participated. Thirty-six (59%) of laboratories scored maximum for genotyping. Nine laboratories (15%) failed to detect sequence variants. The quality of the raw data produced was good. This scheme now gives laboratories an opportunity to compare their performance and use EQA to help and improve the quality of their DNA sequencing.

Molecular analysis of the MECP2 gene in Rett syndrome, Angelman-like and nonsyndromic mental retardation phenotypes. *R. Polli, L. Anesi, A. Casarin, A.M. Laverda, P. Drigo, A. Murgia.* Dept Pediatrics, Univ Padua, Padua, Italy.

Mutations of the Methyl-CpG-binding protein (MECP2) gene, known to be the main cause of Rett syndrome have also been described in individuals with partially overlapping phenotypes. We report the results of a molecular analysis of the MECP2 gene in subjects with neurodevelopmental conditions suggestive of Rett syndrome, in patients with Angelman-like phenotype without alterations of the PWS/AS critical region on 15q11-13, and in subjects with unexplained non syndromic mental retardation. The molecular strategy originally adopted for the study included PCR/SSCP/Sequencing for identification of point mutations or small-size deletions/insertions. This type of analysis allowed the identification of 14 different MECP2 alterations 8 of which disease-causing and 6 polymorphic variants. We found disease-causing mutations in 9/18 female subjects referred for suspected Rett syndrome and in 1/57 individuals with a suspected Angelman syndrome. No MECP2 pathologic variants were identified in 33 subjects with isolated mental retardation. No new disease-causing mutations have been identified, and only two of these are among the most common MECP2 variants. Two of the polymorphisms detected, both in the 3'UTR region, are new variants. Recently a new protocol of Real-Time quantitative PCR, has been implemented and applied to 11 MECP2 negative samples. The new method has allowed to detect, in one female subject with Angelman-like phenotype, the presence of a large deletion that we have been able to document only as a gene dosage reduction on genomic DNA. The fact that only 50% of our clinically Rett syndrome cases resulted to carry MECP2 pathogenic mutations could at least partially be due to the clinical criteria adopted for the selection of the patients. On the other hand, it is likely that MECP2 large genomic deletions/rearrangements are more frequent than previously thought and play a much more relevant role in the aetiopathogenesis of Rett syndrome and other neurodevelopmental diseases. The introduction of new and more precise techniques for the detection of these alterations will probably significantly improve the rate of mutation detection in the MECP2 gene.

Analysis of common alpha-thalassemia point mutations and deletions by reverse-hybridization. *H. Puehringer*¹, *E. Baysal*², *H. Najmabadi*³, *W. Krugluger*⁴, *C. Oberkanins*¹. 1) ViennaLab Labordiagnostika GmbH, Vienna, Austria; 2) Genetics Department, Al Wasl Hospital, Dubai, UAE; 3) Genetics Research Center, The Social Welfare and Rehabilitation Sciences University, Tehran, Iran; 4) Department of Clinical Chemistry, Municipal Hospital Rudolfstiftung, Vienna, Austria.

Alpha-thalassemia (alpha-thal) is the most common inherited hemoglobin disorder worldwide. It is observed in high frequencies throughout Southeast Asia, India, the Middle East, parts of Africa and the Mediterranean area. Alpha-thal is characterized by the reduced synthesis or absence of alpha-globin chains due to mutations affecting one or both genes located in tandem on chromosome 16. Unlike the prevalence of point mutations in beta-thal, the majority of alpha-thal alleles are derived from single gene deletions (-alpha3.7, -alpha4.2) or from double gene deletions (--alpha20.5, --MED, --SEA, --THAI, --FIL). The clinical phenotype varies from asymptomatic to lethal (Hb Barts hydrops fetalis syndrome) according to the number of affected alpha-globin genes and the resulting imbalance of hemoglobin subunits. We have developed a reverse-hybridization assay (Alpha-Globin StripAssay) for the rapid and simultaneous detection of common alpha-globin point mutations, single gene and double gene deletions. The test is based on multiplex DNA amplification (including gap-PCR) and hybridization to teststrips presenting a parallel array of allele-specific oligonucleotide probes for each variant. The entire procedure from blood sampling to the identification of mutations requires less than 6 hours, and hybridization/detection may be carried out manually or essentially automated using existing instrumentation (e.g. TECAN profiBlot). The test is simple and convenient, and requires very small amounts of samples, which is of particular importance for prenatal diagnosis. Although the spectrum of alpha-thal mutations is known to be highly population-specific, the broad range of variants covered by the Alpha-Globin StripAssay should make it a globally useful diagnostic tool. (oberkanins@viennalab.co.at).

Willingness to pay for hereditary breast cancer testing: variation by exposure to Direct-to-Consumer advertising.

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Background: In September 2002, Myriad Genetics, Inc. initiated a direct-to-consumer advertising (DTC-ad) campaign associated with its BRCA 1/2 genetic test in Denver and Atlanta. Genetic testing is a covered benefit in many managed care organizations, but uncertainty exists about the value to members. **Purpose:** To employ willingness-to-pay (WTP) methodology to assess the value of genetic testing services for breast cancer among female MCO members, and compare such values by exposure to the DTC-ad. **Methods:** Written surveys were mailed to a random sample of women aged 25-54 at Kaiser Permanente Colorado (KPCO) in Denver, where the DTC-ad took place, and at Henry Ford Health System in Detroit, where there was no DTC-ad. The survey included WTP questions that varied both with respect to underlying 15-year breast cancer risk as well as bidding algorithms. Recipients were asked for their WTP directly for a genetic test for breast cancer risk, as well as for increasing their monthly insurance premium. **Results:** Overall response rate was 47% with 63% of respondents reporting exposure to information regarding BRCA 1/2 testing; 45% reporting exposure to DTC-ad, with 72% being KPCO members. No significant level of correlation was found across SES variables. Controlling for SES and risk scenario, respondents exposed were 66% more likely than those not exposed to be willing to pay a positive amount out of pocket for testing, with 60% willing to pay \$200, and 24% willing to pay \$500. Of those willing to pay an increased monthly insurance premium, 27% would pay \$15 more per month, 21% would pay \$5 more. No significant start point bias was found. **Conclusion:** Exposure to information regarding genetic testing along with a woman's perceived underlying cancer risk may positively influence her WTP for genetic testing services. Given that testing may only provide value for small percentage of the general population, these results could support variation in co-pay and co-insurance by risk status.

Semi-automated, high throughput fluorescent comparative polymerase chain reaction (FC-PCR) determination of -globin gene dosage: implication for population screening of alpha thalassemia deletions. *S. Wang¹, F. Quan¹, R. Tseng², W. Sun¹, C.M. Strom¹.* 1) Dept. of Molecular Genetics, Quest Diagnostics Nichols Inst, San Juan Capistrano, CA; 2) Dept. of Hematology & Oncology, Quest Diagnostics Nichols Inst, San Juan Capistrano, CA.

Alpha thalassemia is caused by deficient synthesis of -globin, encoded by nearly identical genes, 1 and 2. Over 95% of -thalassemia alleles are caused by deletions involving either or both of the genes. More than 30 different -thalassemia deletions have been reported. The severity of -thalassemia is inversely correlated to quantity of normal -globin genes. Co-inheritance of an -thalassemia allele can ameliorate the phenotypes of -thalassemia, whereas amplification of -globin gene may exacerbate the severity of -thalassemia. To determine the gene dosage, the one-tube FC-PCR amplifies 6 gene fragments by 4 fluorescent primer pairs: **1)** Upstream fragments of both 1 and 2 genes (differ in size by 7-bp) are amplified by same primer pair; **2)** Downstream fragments of both 1 and 2 genes (differing in size by 8-bp) are amplified by second primer pair; **3)** FACC and HEXA genes are analyzed as controls. PCR products are analyzed on an automated DNA analyzer and fragment size and signal intensity analyzed by GeneMapper software. -Globin gene dosage is determined by the collective analysis of fluorescent signal ratio of all amplicons. The assay accuracy was validated by blinded analyses of 44 DNA samples with various copy numbers (0-5) of genes with 100% accuracy. To evaluate utility for population screening of -thalassemia deletion, DNA samples from 88 unrelated individuals, presumably representing diverse ethnic background of US population, were studied. A total of 9 alleles carrying deletion of one -globin gene were detected, representing the carrier frequency of more than 10%. The high throughput and cost effectiveness of this FC-PCR method make population screening for -thalassemia deletions a possibility. In addition to the postnatal diagnosis of thalassemia and evaluation of phenotypic variation of -thalassemia, this method is extremely valuable in prenatal diagnosis of Hb Bart's and Hb H diseases.

Molecular genetic testing for PTPN11 gene mutations in Noonan syndrome. *J. Wiszniewska, A. James, J. Lescher, K. Hoon, P. Fang, M. Hegde, B.B. Roa.* Baylor Medical Genetics Laboratories, Department of Human and Molecular Genetics, Baylor College of Medicine, Houston, Texas, USA.

Noonan syndrome (NS) is an autosomal dominant disorder with an estimated prevalence of 1 in 1,000 to 2,500. Clinical characteristics include facial dysmorphism, growth retardation, skeletal malformations and congenital heart disease as the most common features. NS is genetically heterogenous, wherein mutations in the PTPN11 gene encoding the non-receptor protein tyrosine phosphatase SHP-2 account for approximately 50% of both familial and sporadic cases. Our laboratory implemented clinical molecular testing by sequence analysis of the entire PTPN11 coding region. Analysis of 15 coding exons and the flanking exon/intron boundaries of the PTPN11 gene were performed through PCR amplification and bi-directional DNA sequencing. We studied a cohort of 94 patients referred to our laboratory for Noonan syndrome molecular testing. This group included 5 patients who were indicated to have a definite diagnosis of Noonan syndrome, 65 patients referred with a possible diagnosis, and 24 patients for whom minimal information was provided by the various referral sources. Mutations in the PTPN11 gene were found in a total of 25 out of 94 patients; these were comprised of 1, 19 and 5 mutation positive patients belonging to the first, second and third groups, respectively. Nineteen different mutation alleles were identified. These included two missense variants, 1472CT (P491L) and 1529AC (Q510P), which are allelic substitutions for two previously reported PTPN11 mutations. In addition, we found a novel variant, 155CT (T52I), whose clinical significance is currently undefined. Parental analysis was recommended in these cases. The majority (84%) of mutations were found in exons 3, 8 and 13. Our diagnostic testing experience demonstrates the value of PTPN11 mutation analysis in patients for whom a diagnosis of Noonan syndrome is being considered. These results are consistent with the phenotypic variation that has been reported among patients with PTPN11 mutations.

A novel mutation screening protocol in patients with Wiskott-Aldrich Syndrome (WAS) and related disorders. *K. Zhang¹, D.J. Marmar², A.H. Filipovich², R.J. Wenstrup¹*. 1) Division of Human Genetics, Children's Hospital, Cincinnati, OH; 2) Division of Hematology/Oncology, Children's Hospital, Cincinnati, OH.

Wiskott-Aldrich syndrome is an X-linked recessive disorder characterized by thrombocytopenia, eczema and immunodeficiency. Without bone marrow transplantation and other supportive treatments, median survival is approximately fifteen years of age. WAS is caused by mutations in the gene encoding an intracellular protein, WASP, that is involved in signal transduction and regulation of actin cytoskeleton organization of hematopoietic cells. The gene responsible for WAS has 12 exons, encodes a 502 amino acid proline-rich protein. To date, about 170 mutations have been reported in the WAS gene, resulting in classical WAS and several other leukopoietic disorders including X-linked thrombocytopenia (XLT) and X-linked congenital neutropenia (XLN). These mutations are broadly distributed through all the exons. Linkage analysis, single-strand confirmation polymorphism (SSCP) mutation screening, and X-chromosome inactivation studies have been used to identify the mutations in WAS. However, given the size and molecular composition of the WAS gene, these protocols are either labor intensive or lack the sensitivity which is required in a clinical setting. We developed a PCR and direct-sequencing based protocol for WAS mutation screening. A single PCR program was designed and optimized to amplify the entire coding region and all twelve exon/intron boundaries at once. The PCR products were then used to perform sequencing analysis. This protocol requires only 4 hours of technician time and approximately one day of machine time. Six patients and/or obligate carriers were tested by this method. Two novel mutations have been identified. The results are supported by intracellular WAS protein analysis in leukocytes using a monoclonal antibody by flow cytometry. This new protocol has been shown to be reliable, sensitive, specific and cost effective. It provides the opportunity for physicians to make timely diagnosis of WAS.

Maternal Serum Screening in Northern Alberta: The Need for a Provincial Program. *K.J. McElligott¹, S.M. Christian¹, S.A. Kieffer², J. Reeve³, F. Bamforth³, J.S. Bamforth³.* 1) Medical Genetics, Alberta Children's Hospital, Calgary, Alberta, Canada; 2) University of British Columbia, Vancouver, British Columbia, Canada; 3) University of Alberta, Edmonton, Alberta, Canada.

Background: Utilization of maternal serum screening in Alberta is significantly lower than in other provinces in Canada with structured screening programs. **Method:** In an effort to understand this low utilization rate, knowledge, attitudes and practice patterns of physicians in Northern Alberta were assessed through a survey. **Results:** This survey found that only 22 % of physicians were appropriately offering the screen to all their pregnant patients. Physician age, number of prenatal patients followed, and whether the physician felt that the test was standard of care and medically-legally necessary to offer, significantly influenced practice patterns. Finally, physician views on termination also significantly influenced practice patterns. **Interpretation:** The under utilization of a test that is offered as standard of care across Canada places Alberta patients at a disadvantage. One avenue, which has been adopted in other provinces, is the development of a provincial screening program. By way of a comprehensive program, educational issues, and appropriate counselling and follow up which are current barriers to the provision of care, can be addressed.

Efficient mutation detection in Hereditary Hemorrhagic Telangiectasia using Temperature Gradient Capillary Electrophoresis, Conformation Sensitive Gel Electrophoresis, and High-Resolution Melting Analysis. *P. Bayrak Toydemir^{1,2}, L-S. Chou¹, J.G. Vandersteen², F. Gedge¹, C.T. Wittwer^{1,2}, E. Lyon^{1,2}, R. Mao¹.* 1) Associated Regional & University Pathologists, Inc.(ARUP), Salt Lake City, UT; 2) Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT.

Hereditary Hemorrhagic Telangiectasia (HHT) is an autosomal dominant vascular dysplasia with an estimated frequency of 1-2 in 10,000. Affected individuals have epistaxis, mucocutaneous telangiectases and visceral arteriovenous malformations. Two molecular sub-types are now recognized: HHT1 is caused by mutations in the endoglin gene (*ENG*) and HHT2 is caused by mutations in the activin receptor-like kinase 1 gene (*ALK1*). Although mutations in these genes account for more than 90% of HHT cases, developing simple and reliable diagnostic approaches has been difficult because of the lack of common alleles. Thus, sensitive mutation scanning approaches followed by targeted sequencing might be useful in clinical settings.

Many scanning techniques have been developed to detect mutations, which differ in sensitivity, specificity, throughput, and cost. In this study, we evaluated three mutation screening techniques, all based on heteroduplex formation: Temperature Gradient Capillary Electrophoresis (TGCE), Conformation Sensitive Gel Electrophoresis (CSGE), and High-Resolution Melting Analysis (HRMA).

We studied 15 HHT cases with a confirmed clinical diagnosis and known mutations. Mutation scanning by TGCE, CSGE and HRMA was performed for the entire coding region and intron/exon boundaries of both *ALK1* and *ENG* genes. The sensitivity of CSGE, TGCE, and HRMA techniques for identifying HHT mutations were 63.64%, 93.33%, and 100%, respectively. We have found that both TGCE and HRMA are favorable mutation scanning techniques to identify the mutations responsible for HHT.

How effective are clinical genetic services? *M. McAllister¹, K. Payne¹, L. Davies², S. Nicholls¹, R. MacLeod¹, H. Middleton-Price¹, D. Donnai¹.* 1) Nowgen, St Mary's Hospital, Manchester, United Kingdom; 2) HER@M, The University of Manchester, Manchester, United Kingdom.

There is a lack of appropriate instruments to measure the benefit of clinical genetic services (CGS). The nature of a good outcome is not clearly understood and outcome measurement is problematic since patients are generally healthy. This study forms part of the Nowgen research programme and aims to identify users and service providers views about the positive and negative effects of genetic conditions on individuals and families and compare these to existing measures used to evaluate genetic services. Focus groups were used to identify key aspects of health and social well-being that are affected by genetic conditions, and CGS (outcomes). The focus groups were run by a trained facilitator and audio and video recorded. The findings were transcribed and analysed using the constant comparative method. A systematic literature review is being used to evaluate the relevance and usefulness of instruments to evaluate CGS. Initial results from the focus groups indicate positive and negative effects that genetic diseases have on individuals and families: emotional, that may be influenced by culture, socio-economic status and gender (eg. guilt, worry, family disruption; practical (eg. financial burden and reduced life choices) and physical (eg. mobility problems and lifelong care). An initial review of outcome measures used to evaluate CGS indicates that (i) no single measure encompasses all the attributes that were identified by the focus groups as important to service users and (ii) that there are insufficient valid and reliable instruments to measure all the attributes. Further work is required to develop an array of instruments to measure all relevant attributes and weight them according to their importance.

Valuing preferences for a clinical genetic service. *K. Payne¹, M. McAllister¹, H. Middleton-Price¹, D. Donnai¹, L. Davies².* 1) Nowgen, St Mary's Hospital, Hathersage Road, Manchester, UK; 2) HER@M, The University of Manchester, Manchester, UK.

Discrete choice experiments (DCEs) are a type of survey instrument that have been used successfully to value peoples preferences for health care services but, to date, not used widely in the valuation of genetic services. The method is rooted in economic theory (random utility theory), which closely reflects how we make decisions about which products to choose and use every day. The theory assumes that the total value (utility) someone attaches to a product (service) is described by the sum of individual attributes (or characteristics). These attributes can relate to the clinical outcome of the service (eg. accuracy of risk information) or the process of providing the service (eg. who provides the service). The ability to incorporate outcome and process attributes is one of the advantages of this method. The attributes are then described by levels, which indicate the different values the attribute can take. DCEs estimate the individual effect these attributes have on a population of peoples preferences for a service. The theoretical basis of DCE assumes that consumers make trade-offs between the attributes. A vital step in the design of any outcome measure is detailed exploration of what factors (or attributes) are/should be considered when describing the processes of service delivery and the outcomes. The aim of this paper is to illustrate how DCEs may be used as a robust valuation tool to provide a measure of service users and providers preferences and values for process and outcome attributes associated with the delivery of clinical genetic services. The paper will also describe how the results of focus groups and systematic reviews completed as part of the Nowgen research programme can be used to inform the design of a DCE.

Resequencing on a Chip: Is it ready for prime time? A. Ganguly¹, C. MacMullen², C. Stanley². 1) Department of Genetics, University of Pennsylvania, Philadelphia, PA; 2) Division of Endocrinology, The Childrens Hospital of Philadelphia.

Resequencing of multiple large genes in parallel can be achieved by using high density oligo-nucleotide microarrays. We summarize our experience of resequencing using Custom-Seq chips synthesized by Affymetrix, CA in the context of Congenital Hyperinsulinism (HI). HI, the most common cause of persistent hypoglycemia in infants, is caused by mutations in 4 genes: the KATP potassium channel comprised of the sulfonylurea receptor (SUR1) and inwardly rectifying ion pore (Kir6.2), glutamate dehydrogenase (GDH), and glucokinase (GK). Children unresponsive to medical therapy undergo pancreatectomy. The extent of pancreatectomy correlates to the mutation status of the SUR1 and Kir6.2 genes. We designed the HI-CHIP for detecting variations in the 65 coding exons including flanking intronic regions of the 4 HI genes. We evaluated the resequencing method using genomic DNA from 13 individuals: 1 negative control, 2 positive controls, and 10 unknowns, and compared the results to conventional automated fluorescent sequencing. Initial base call rates averaged 92% (9393bp/10210bp) with a range of 61 to 96%. This translates to an average of 12 n calls per tiled exon. Under relaxed settings for the analysis software, the average base call rate improved to 96% (9851bp/10210bp) with a range of 84- 98%. This resulted in an average of 6 n calls per exon. About 60% of the amplicons had to be directly sequenced after scanning due to presence of undefined n calls within possible exonic and/or splice sites. The sequence context of these n's is predominantly a stretch of Cs (>3 in a row). Visual inspection of the probe hybridization data indicate the possibility of better base calls if the algorithm could be trained to look at the data from one strand only. The method has yet to be optimized to perform as well as direct sequencing for any custom set of genes. In conclusion, resequencing on HI-CHIP has the potential to generate HI genotypes efficiently. In future, HI-CHIP can be a clinically useful pre-operative diagnostic tool to determine the extent of pancreatectomy in medically unresponsive HI cases.

Sensitive RNA/DNA mutation detection for dystrophinopathies through uniform direct sequencing. *A.L.J. Kneppers, D.J. Verbove, A.S. Hoogenboom, E. Bakker, H.B. Ginjaar.* Center for Human and Clinical Genetics, Leiden University Medical Center, P.O.Box 9503, 2300 RA Leiden, the Netherlands.

In about one third of dystrophinopathy patients small mutations in the large dystrophin gene are identified as the disease causing defect. Different mutation detection techniques on both RNA and DNA level are used to scan for such mutations. Besides a large number of nonsense mutations the mutation spectrum for small mutations also shows mutations affecting splicing. Along with splice site acceptor and donor mutations deep intronic mutations leading to a splicing artefact are also found in patients. Dystrophin RNA studies are needed to detect this type of mutations and therefore should be included in a mutation scanning protocol for dystrophinopathies. For the exact identification of all small mutations and subsequent family studies the gold standard remains the direct sequencing of the fragment containing the mutation. A standardized procedure using M13 tailed exon specific primers allows direct sequencing using the M13 sequence primers for all PCR fragments in a uniform manner. We have developed, optimised and validated a uniform direct sequencing protocol for dystrophin gene analysis of both the coding sequence on mRNA level as well as all 79 exons including splice sites on the genomic level. Starting with RNA isolates of lymphocytes or muscle biopsies 20 overlapping PCR fragments are generated in 2 subsequent PCR reactions after cDNA synthesis. For the genomic analysis 78 exon specific fragments are generated. The procedure can be automated for PCR and sequence reaction set-up. All PCR fragments are sequenced for both forward and reverse strand and analysed in a semi-automated fashion after data collection. So far mutation scanning in 97 patients revealed a mutation in 94 % of all cases. Classification of the mutations showed 38 % nonsense, 27 % frame shift and 27 % splice site mutations of which 3 % appeared to be deep intronic mutations. This uniform sequencing approach complements the initial deletion/duplication scanning in dystrophinopathy patients, enables a standardized procedure for mutation scanning and has been successfully applied to other genes.

Program Nr: 1510 from the 2004 ASHG Annual Meeting

CFTR gene mutations in Iranian cystic fibrosis patients: Detection of

F508, G542X, W1282X, N1303K, A120T, R117H and R347H mutations. *F. Mirzajani¹, R. Mirfakfraie¹, M. Jalalirad¹, N. Yazdanpanahi², M. Mojarad², Gh. Babamohammadi¹, E. Talachian³, M. Rafiei⁴, P. Derackshandehpeikar², M. Goharbari¹, M. Houshmand¹.* 1) Medical Genetics Dep., National Institute of Genetic Engineering & Biotechnology, Tehran, Iran; 2) Tehran Medical University, Tehran, Iran; 3) Aliasghar Hospital, Tehran, Iran; 4) Children Medical Center, Tabriz, Iran.

50 unrelated CF families were screened for the most common mutations including F508, G542X, W1282X, N1303K and G551D in the CFTR gene using ARMS-PCR method. Exons 4, 7, 10 and 11 were screened for any other possible mutations using SSCP-Sequencing method. F508 mutation covered only 18% of the mutated alleles, which is one of the lowest frequencies detected worlds wide so far. G542X, W1282X and N1303K mutations accounted for 8%, 8% and 2% of the mutated alleles respectively. SSCP-Sequencing method also revealed the occurrence of R117H, R347H and A120T mutations in the studied population..

Shwachman-Diamond Syndrome: one year Experience in a Molecular Diagnostic Laboratory. *L. Steele¹, J. Rommens², N. Bulgin¹, Y. Yang¹, L. Han¹, M. Eliou¹, T. Wright¹, P.N. Ray^{1,2}, T. Stockley¹.* 1) Dept Paed Lab Med; 2) The Research Institute, The Hosp for Sick Children, Toronto, ON, Canada.

Shwachman-Diamond Syndrome (SDS) is an autosomal recessive disorder with an incidence of 1 in 50,000 births. The primary features of SDS include bone marrow problems, a defect in the pancreas, skeletal abnormalities, and short stature. The SBDS gene at 7q11 has recently been found to be the causative gene for SDS. A pseudogene, SBDS-P, exists within a distally duplicated region and shares 97% nucleotide sequence identity with the SBDS gene. A majority of SDS patients have mutations resulting from recombination and gene conversion between the SBDS and SBDS-P genes. The two common conversion mutations are reported to account for 75% of all SDS disease alleles. Here we report our experience with molecular genetic testing for SDS in a clinical diagnostic laboratory at the Hospital for Sick Children. The strategy consists of two stages: A direct mutation detection assay is used to test proband samples for the two common mutations in exon 2 of the SBDS gene, 183_184TA->CT and 258+2t->c. A proband is expected to have one of these common mutations 90% of the time. If one mutation is found, samples would undergo direct sequencing of all exons and intron/exon boundaries for a rare mutation in the SBDS gene. Approximately 31% (11/35) of probands referred were confirmed by molecular testing to have the diagnosis of SDS; 82% (9/11) of these had two common mutations whereas 18% (2/11) had a common and a rare mutation. None of the probands without a common mutation who also had full SBDS gene sequencing (31%) were found to have a rare mutation (11/35). Parental samples, if available, were useful in distinguishing whether the two common mutations were in cis or in trans. Prenatal diagnosis has been performed on 5 cases to date and predicted a favorable outcome in all cases. An accurate diagnosis can now be made in families affected with Shwachman-Diamond Syndrome and prenatal diagnosis is available as an option for these couples who are confirmed carriers.

Program Nr: 1512 from the 2004 ASHG Annual Meeting

Extensive Mutational Analysis in Duchenne/Becker Muscular Dystrophy. *T.L. Stockley, S. Akber, L.K. Steele, P.N. Ray.* Molecular Genetics Laboratory, Hospital for Sick Children, Toronto, ON, Canada.

Duchenne and Becker muscular dystrophies (D/BMD) are neuromuscular disorders caused by mutations in the dystrophin gene. It is reported that 65% of mutations are partial gene deletions, 5% are partial gene duplications and 30% are point mutations. Our laboratory has developed extensive molecular diagnostic assays for detection of mutations in the dystrophin gene. These assays include (1) quantitative PCR multiplex assays for all 79 exons of the dystrophin gene and the brain promoter to detect partial gene deletions and duplications and (2) complete sequencing of all exons of dystrophin and intron/exon boundaries from genomic DNA to detect point mutations. These assays are predicted to detect the majority of mutations in dystrophin causing D/BMD. We have applied these assays to detection of mutations in an unselected sample of 187 males referred for D/BMD testing to our clinical molecular laboratory from 1990 to 1994 inclusive. All samples were tested initially using the complete multiplex analysis for deletions/duplications in all exons. 110 males were found with partial gene deletions and 15 males were found with partial gene duplications. A significant number of single exon deletions (20/110) and duplications (7/15) were identified. Of deletions and duplications identified, 9% of deletions and 25% of duplications would be missed by current protocols using multiplex analysis of 18 dystrophin exons. The 62 males without deletions or duplications were further assessed for sequencing. 10 samples were unlikely D/BMD on review of clinical information. The remaining 52 samples underwent sequencing of the dystrophin coding region, and 14 samples have been identified with point mutations to date. The remainder of samples without identified mutations are being further analyzed. This study has implications for refining the frequency of mutations causing D/BMD.

Australian views on commercial and government interests in genetic test results and implications for public policy. *K. Barlow-Stewart¹, S. Treloar², S. Taylor², M. Saleh¹, I. Muchamore¹, M. Stranger³, M. Otlowski³.* 1) Ctr Genetics Education, Royal North Shore Hosp, Sydney, Australia; 2) Scl Soc Wk, Univ Queensland, Brisbane, Australia; 3) Scl Law, Univ Tasmania, Hobart, Australia.

Aim: To study Australian views on current and future use of genetic information in life insurance, employment and immigration and support for genetic testing overall. **Method:** A new biennial mailed, self-completed Australian Survey of Social Attitudes was sent to 5,000 randomly selected people on the 2002 Australian electoral role in 2003 by the Australian National University. 20 questions on human genetics were included with a 41.7% response rate. **Results:** 45% of respondents linked inherited health problems with human genetic information, providing a measure of genetic health awareness. 49% correctly think genetic test results are currently used in life insurance underwriting. 58% think it is not currently used in employment which appears to be the case in Australia. 77% disagree with insurance companies being able to genetically test policy applicants in the future and 75% disagree with its use in the workplace, even in the context of safety for both current and future employees. While over 30% agree with genetic testing potential immigrants for future health problems, their support is closely linked to other attitudes about migrants. One in three who disagree with genetic testing on themselves in insurance or employment support its use on others who wish to migrate to Australia. Nevertheless twice as many respondents strongly disagree with genetic testing of immigrants for future health problems than strongly agree. 66% support genetic testing overall, as opposed to 17% with concerns about its misuse and 17% found this choice too hard to make. The main influencing factors on awareness and opinions were respondent's demographic, social and political values rather than their level of genetic health awareness. **Conclusion:** Education is crucial if utilisation of genetic testing is to be on an informed basis. Australians express strong opposition to the use of genetic information by commercial and government interests. Public policy must therefore ensure its use is monitored and regulated.

Reproductive genetic testing: Options for policymaking. *S. Baruch, K. Hudson, G. Javitt.* Genetics and Public Policy Ctr, Johns Hopkins University, Washington, DC.

An array of medical procedures and genetic tests can inform individuals about possible outcomes of current or future pregnancies. These tests and procedures, including carrier testing, preimplantation genetic diagnosis, and prenatal testing, can determine whether a genetic alteration is present that is likely to cause a specific disease or condition. As our knowledge of the human genome increases, the number of conditions for which testing is possible will increase accordingly, as will legal and ethical questions.

Current government oversight of reproductive genetic testing is limited and confusing. This presentation will describe the current state of regulation. Various federal and state entities have asserted authority over some aspects of reproductive genetic testing, but gaps remain. Federal and state policymakers are considering whether additional oversight is needed to guide the use and development of reproductive genetic technologies.

The Genetics and Public Policy Center has developed a range of innovative policy options, informed by extensive social science research and outreach to stakeholders. Policy preferences are shaped by a variety of factors, including an individual's expectations of existing and future applications of tests, core beliefs about the moral and ethical impact of testing, assumptions about the expected costs and benefits and how they will be distributed, and fundamental views about the proper balance between government involvement and individual liberty.

The options presented will reflect the range of perspectives revealed in our research and will include the strongest arguments for and against each option. Options include approaches for federal and state oversight, as well as self-regulation by professional organizations. Options respond to a range of concerns about the safety and accuracy of tests, whether they are being offered and performed at the right time, what diseases or traits patients are testing for, and what impact reproductive genetic testing may have on individuals, families, and society.

Setting research priorities to support policy making in genetics. *I. Blancquaert¹, G. Cleret de Langavant¹, W. Foulkes², D. Gaudet³, B. Godard³, N. Laflamme⁴, A. Marcoux⁵, F. Rousseau⁶, R. Battista³, APOGEE-Net Network.* 1) Genetics Unit, Agence d'évaluation des technologies et des modes d'intervention en santé (AETMIS), Montreal, Quebec, Canada; 2) McGill University, Montreal, Quebec, Canada; 3) Université de Montréal, Montreal, Quebec, Canada; 4) Institut National de Santé Publique (INSPQ), Montreal, Quebec, Canada; 5) Conseil de la Santé et du Bien-être (CSBE), Québec, Quebec, Canada; 6) Université Laval, Québec, Quebec, Canada.

More trans-disciplinary policy-oriented research is needed to ensure that the wealth of knowledge that is produced in genetics be both useful and usable by policy makers in their efforts to implement a judicious technology transfer in genetics. In addition, an active strategy of knowledge translation is essential to achieve such a goal. APOGEE-Net is a knowledge network that brings together producers, disseminators and users of knowledge to support policy making in the area of genetics. More specifically, university based researchers in a variety of disciplines, researchers from organizations whose mandate is to counsel decision makers and to promote knowledge translation, clinicians, public representatives and policy makers from five Canadian provinces participate. Knowledge transfer, trans-disciplinary research and capacity building (research and receptor) are key objectives of the network. The original knowledge transfer strategy rests on iterative interactions between decision makers and researchers at different stages of the research cycle : in setting research priorities and in the interpretation of research results. These exchanges provide an opportunity to progress towards a shared understanding of the challenges and issues related to the application of new genetic technologies. This communication describes the first priority setting exercise, based on a combination of a Delphi survey and a Nominal group technique, both adapted to the specifics of the APOGEE-Net network. Priorities in all three research axes were identified: genetics and health services, genetics and public health and genetics and citizen dialogue.

Program Nr: 1516 from the 2004 ASHG Annual Meeting

Developing a Sustainable Process to Provide Quality Control (QC) Materials for Genetic Testing. *B. Chen¹, D.J. Boone¹, L. Silverman², I. Lubin¹, J. Amos³, J. Beck⁴, M. Chan⁵, D. Farkas⁶, R. Lebo⁷, C. O'Connell⁸, C.S. Richards⁹, B. Roa⁶, The QC Materials for Genetic Testing Group.* 1) Ctrs for Disease Control & Prevention; 2) Univ of Virginia; 3) Specialty Laboratories; 4) Coriell Inst for Medical Research; 5) Food and Drug Administration; 6) Baylor College of Medicine; 7) Childrens Hospital Medical Ctr of Akron; 8) National Inst of Standards & Technology; 9) Oregon Health & Science Univ.

The quality of genetic testing services can be compromised by the lack of available QC materials. Therefore, in September 2003 and March 2004, the Centers for Disease Control and Prevention organized two QC Materials for Genetic Testing conferences, to develop recommendations for practical, sustainable mechanisms for making QC materials available to the genetic testing community at a reasonable cost. More than 50 leaders in genomics and genetic testing participated in the conferences and formed subcommittees to address the following needs: 1) determining genetic tests in the greatest need of appropriate QC materials, 2) developing networks of material contributors, 3) using existing cell banks as material sources, 4) facilitating research collaboration, 5) developing validation processes, 6) determining needs for professional guidance, 7) clarifying regulatory oversight, and 8) improving funding support. The progress, ongoing activities, and further recommendations from the group will be presented. Among the recommendations for future projects was identifying a National QC Materials Coordinator to provide continuing support and coordination for development and provision of QC materials. Proposed activities include: 1) facilitating development, collection, submission, validation, and distribution of QC materials; 2) monitoring community needs; and 3) improving information exchange among users, repositories and manufacturers of control and reference materials. A third QC Materials for Genetic Testing conference will be held in November 2004 to further develop project plans and obtain additional input. Information regarding the QC material activities is available at <http://www.phppo.cdc.gov/mlp/qcgeneticconference/proceedings.asp>. **QC Materials for Genetic Testing.**

Carrier Screening for Recessive Diseases: Reasons for Caution about Cystic Fibrosis Carrier Screening. *A.M. Laberge, K. Porter, W. Burke.* Institute for Public Health Genetics and Department of Medical History and Ethics, University of Washington, Seattle, WA.

Using key informant interviews and literature review, we evaluated the policy history of carrier screening for Tay-Sachs disease (TSD) and other genetic disorders prevalent in the Jewish community, beta-thalassemia (BT), and cystic fibrosis (CF). TSD and BT screening are considered highly successful. Their features include: 1. Severe disease; 2. Limited phenotypic variability; 3. Lack of treatment; 4. High test sensitivity; 5. Availability of prenatal diagnosis. Both TSD and BT screening programs are also characterized by community involvement, support of parents groups, and evidence of a strong consensus in favor of avoiding affected births. Initial programs for TSD in the US and for BT in Cyprus and Sardinia were rapidly replicated and have significantly reduced disease incidence within screened communities. By contrast, because of wide phenotypic variability and availability of treatment, a lack of consensus is currently seen about the inclusion of Gaucher disease in expanded Jewish panel carrier tests, in spite of community support for carrier screening in general. In this context, NIH (1997) and ACOG/ACMG (2001) recommendations for population-based CF carrier screening raise concerns. CF does not share the features of TSD and BT: it has wide phenotypic variability, available treatments have significantly improved life expectancy and quality of life, and testing is complex. Test sensitivity varies greatly by ethnicity, results can be difficult to interpret, and phenotype is hard to predict from genotype. Furthermore, the Cystic Fibrosis Foundation does not identify CF carrier screening as a part of its mission, and current Canadian practice guidelines specifically state that the offer of CF carrier screening does not constitute a standard of prenatal practice. Like Gaucher disease, the need to avoid CF is debated because of wide phenotypic variability and increased life expectancy. Together, all these factors point to a lack of consensus about CF carrier screening and the need for caution in promoting it.

Identification and characterization of patients at-risk for hereditary breast cancer in a community-based study in Southern Brazil. *P. Ashton-Prolla*^{1,2,3,4}, *M. Caleffi*⁴, *E.I. Palmero*³, *L. Kalakun*¹, *R. Giugliani*^{1,2,3}, *L. Schuler-Faccini*^{1,2,3}. 1) Service of Medical Genetics - HCPA; 2) Department of Genetics - UFRGS; 3) Postgraduate course in Genetics and Molecular Biology - UFRGS; 4) Hospital Moinhos de Vento, Porto Alegre.

Hereditary breast cancer (HBC) accounts for a minority of breast cancer cases (BC) in most populations, but the identification of at-risk patients is important because they have a higher lifetime risk of BC and other tumors; other at-risk relatives may be identified, and, effective prevention strategies are available. In Brazil, BC is the 1st cause of cancer-related deaths in women of all ages. In Rio Grande do Sul, the southernmost State, BC is the first cause of all deaths in young women (30-49 ys). The states capital, Porto Alegre, has the highest BC incidence and mortality rates in the country. This study will examine the contribution of genetic risk factors to such distinct BC statistics in the area. This question will be addressed through identification and characterization of HBC at-risk patients and families in a sample of 10.000 women residing in a section of Porto Alegre. Women over the age of 15 will be actively recruited by municipal health agents and submitted to an HBC risk-questionnaire that consists of 6 questions to assess presence of a 1st degree relative with BC and/or OC; male relative with BC; relative with BC under 50; 2 or more relatives with BC and/or OC; relative with bilateral BC; relative with both breast and ovarian cancer. If any of these is identified the patient will be invited for genetic counseling and if applicable, genetic testing. Additional questionnaires (cancer knowledge, risk perception, family dynamics) will be applied to all eligible patients. Expected outcomes are determination of specific patient profiles (i.e. regarding HBC knowledge, risk perception, attitudes) and contribution of mutations in each of the specific BC predisposition genes studied (BRCA1, BRCA2, p53, CHEK2). Two months after beginning of the study, approximately 500 women were recruited and about 20 percent reported at least one positive risk factor in the screening questionnaire.

Program Nr: 1519 from the 2004 ASHG Annual Meeting

Nowgen-the North West Genetics Knowledge Park. *D. Donnai*¹, *H.R. Middleton-Price*². 1) Regional Genetics Service, St Mary's Hospital, Manchester, United Kingdom; 2) Nowgen, St Mary's Hospital, Manchester, United Kingdom.

Nowgen (The North West Genetics Knowledge Park) is one of a network of six Genetics Knowledge Parks in the UK initiated and funded by the Departments of Health and Trade & Industry. Nowgen is a partnership between four universities and a teaching hospital in the North West of England. Nowgen's research and work programmes cover three themes: improving the provision of genetic services; improving public engagement and professional education; developing ethical and legal frameworks. In addition, Nowgen: Provides information resources to professionals and the public. Has created a forum for education and dialogue around human genetics. Supports industry by providing training and knowledge transfer in issues relating to human genetic information. Has involved genetics service users and the public in all aspects of its activities. Has developed a sophisticated web based knowledge management system to link and empower communities concerned with human genetics. Has built a new Centre in Manchester, UK the Nowgen Centre (opening late 2004). To date Nowgen's activities include: Contributing to the Manchester Masters programme training genetics professionals. Workshops on: Communication Skills; Disability and Reproductive Choice; Preferences Values and Risk in Genetics Based Health Care. A public debate: GeneHype. The UK network of Genetics Knowledge Parks have complementary strengths and are collaborating on many aspects of their research programmes, on education and training, and on delivering workshops and conferences.

Program Nr: 1520 from the 2004 ASHG Annual Meeting

The Sino-American Genomics Foundation (SAG). *R. Fineman*¹, *A. Chen*². 1) Sino-American Genomics Foundation, Seattle, WA; 2) CDC, Atlanta, GA.

SAG's mission is to improve maternal and child health in developing countries through the practical application of public health genetics and genomics. This expertise, though readily available in the US and other technically advanced countries, is not widely available in the People's Republic of China (PRC), India, and most developing regions of the world. SAG's mission is being carried out in China using simple, practical, cost effective and time-tested public health policies and practices (for example, utilization of the public health core functions) capable of being reproduced in other developing countries in the future. Our initial, infrastructure-building efforts encompass a process (primarily in six locales in China totaling 230 million people) that includes, for example, improvements in China's newborn screening and birth defects surveillance and monitoring systems; a web site in Mandarin and English to facilitate ongoing communications, education, public relations and monitoring of progress; a sister hospital exchange program; and genetics and genomics education programs (including, for example, one for folic acid diet supplementation) aimed at health care professionals, decision-makers, families of affected children, and the lay public with special emphasis on individuals 15-45 years of age. To the best of our knowledge, SAG is the only public health genetics and genomics 501(c)(3)-approved, private, non-profit, foundation of its kind in the US. China is where SAG's efforts will begin, not end. Working together, SAG and its partners in China and the US aim to increase access to genetic and genomic health care services worldwide.

Many prominent individuals in public health and other fields, in both the US and China, have become actively involved in SAG's activities, and they have endorsed SAG's vision of preventing severe mental retardation and birth defects in tens of thousands of babies born in China every year.

Analysis of Inquiries to a Federal Education Resource on Genetic and Rare Diseases. *J. Lewis¹, K. Hagerty¹, L. Lanier², H. Hyatt-Knorr³.* 1) Aspen Systems Corp, Rockville, MD; 2) NHGRI, Bethesda, MD; 3) ORD, Bethesda, MD.

For more than 2 years, the Genetic and Rare Diseases (GARD) Information Center has provided a bridge between information-seekers and reliable resources on genetic and rare diseases. Inquiries to GARD have been collected and analyzed during 32 months of operation. Personal identifiable information obtained in developing responses to inquiries is not retained in any form. The data analyzed are in the aggregate. The National Human Genome Research Institute (NHGRI) and the Office of Rare Diseases (ORD), NIH, launched GARD to provide free and immediate access to reliable information about genetic and rare diseases in English and Spanish. GARD Information Specialists respond to inquiries received through toll-free telephone, TTY, email, fax, and letter. Data analysis of inquiries includes: audience segments and characteristics; inquiry topics, by audience, channel, region and language (English or Spanish); resources requested and provided; trends in volume, by channel and time of year; the impact of outreach efforts on volume. Data have been collected for the 8,000+ inquiries handled by GARD to date. Of the people who provided information about themselves (86%), inquiries came from patients (28%), their families (42%), health professionals (10%), and the general public (20%). Approximately 92% of GARD users request information about a specific disease. With 3000+ disease inquiries received, trimethylaminuria and cystic fibrosis are the top two. Ten percent of inquirers requested information about genetic services, testing, and research. Being aware of the types of information people search for and obtain when they contact GARD will help genetics professionals stay abreast of trends in their clients information-seeking behaviors, help them understand their clients underlying questions and informational needs, and enable them to more effectively structure patient sessions and communicate with allied health professionals. Other stakeholders in genetics (researchers, allied health professionals, and others) might also find it helpful to know how they can use GARD to assist them in connecting people to information and resources about genetic and rare diseases.

Genetic and environmental risk factors in mental retardation: Knowledge, perceptions and attitudes of health professionals. *A.P. Marques-de-Faria¹, A.M.S.M. Moraes^{1,2}, L.A. Magna¹.* 1) Dept. of Medical Genetics, State University of Campinas School of Medicine, Campinas, SP, Brazil; 2) Dept. of Medicine, University of Maringá, PR, Brazil.

As a consequence of advances in medical genetics, demand for clinical genetic services has increased, reinforcing the need for programs towards the management and prevention of genetic diseases and birth defects. Considering mental retardation (MR) as a major problem in clinical genetics, a survey has been developed in Maringá, PR, a 283,792 inhabitants Brazilian town, with good health indicators for public and private care. A total of 156 health care professionals, encompassing 90 physicians (pediatricians, internists, obstetrician-gynecologists, family practitioners) and 66 nurses, were surveyed to assess their knowledge, perceptions and attitudes about genetic and environmental risk factors for MR. In addition, an interview was carried out among 100 women in the puerperal period, all of them recorded as having a healthy newborn. A profile has been delineated and some aspects deserve mention as most of health professionals demonstrating lack of knowledge in relation to issues as epidemiology and MR preventive measures or the national newborn screening program. They also answered not have self-assurance for taking family history and advising about prenatal diagnosis procedures. Although they declared that usually verify MR risk factors occurrence, the majority of women interviewed denied this procedure. Ignorance about genetics, difficulties to carry out complementary studies and refer patients to genetic services, besides the long time required to record medical history, were considered disadvantageous factors in the evaluation of patients with genetic diseases. The improvement of health care providers by the integration of genetics content into the knowledge base of professionals and students of the health professions, as well as the education of general population, are among the respondents suggestions, which also pointed out the presence of a clinical geneticist at the public health service as essential for implementing preventive strategies and for the success of community programs in MR.

Genetic risk assessment of oocyte donors. *M. Topping*¹, *A. Matthews*¹, *S. Kingsberg*^{1,2}, *T. Hassold*¹, *P. Hunt*¹. 1) Case Western Reserve University, Cleveland OH; 2) University Hospitals of Cleveland, Cleveland OH.

The role of genetics in gamete donor evaluation varies among assisted reproductive technology (ART) clinics. To investigate the implementation of the American Society for Reproductive Medicine genetic screening guidelines and examine the potential impact of genetics professionals (geneticists/genetic counselors), in vitro fertilization programs in the Society for Assisted Reproductive Technology (SART) registry were surveyed. The survey included questions on the frequency of screening for recessive disorders in high-risk populations in the absence of family history, how ART clinics handle potential donors with positive family histories (cystic fibrosis, Huntington, cleft lip, mental retardation, breast cancer), and the extent to which genetics professionals are involved in risk assessment. Thirty percent (116/380) of the SART registry participated. Results reported are from 77 ART clinics responsible for genetic risk assessment of potential oocyte donors. While the majority of programs follow standard of care guidelines, many do not. Only 25% of programs karyotype potential donors. Donors are least likely to be tested for Canavan disease (54.2%), -thalassemia (54.4%) and -thalassemia (56.9%) in their respective high-risk ethnic populations. More clinics always screen for cystic fibrosis among Caucasians (77.5%) and Ashkenazi Jews (78.1%), and Tay-Sachs disease (83.3%). In the presence of a positive family history, programs were more likely to exclude donors regardless of the genetic risk involved in order to potentiate the most desirable pregnancy outcome and to maximize the potential of recipients selecting such donors in light of program costs and efficiency. When genetics professionals are involved, the frequency of screening and counseling for genetic risk increases, suggesting that the use of geneticists improves the extent of risk assessment. Therefore, increased focus on genetics in gamete donor selection is warranted, including the utilization of genetics professionals for more consistent and accurate risk evaluation, psychosocial counseling and future guideline development.

National Information Center about Teratogens (SIAT): analysis of its impact over the pregnant women health during its 14 years of service. *L.H. L. Rossi¹, R. Pers¹, M. Sanseverino¹, G. Maegawa², C. Ribas¹, M. Jann¹, S. Costa¹, M. Golbert¹, L. Schuler-Faccini¹.* 1) Medical Genetics Service, Medical Genetics Service, HCPA, Porto Alegre, RS, Brazil; 2) Div. Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto, CA.

The National Information Center about Teratogens (SIAT) was established in 1990 in Porto Alegre, RS as the first Latin American service in this field. The objective is to provide accurate information about teratogenic risks related to chemical, physical or biological agents to which most pregnant women can be exposed. The service is devoted to health care providers and also to the general community. All the information required are filed in a data bank in order to be used for future studies in human teratogenesis and investigate the potential teratogen effect of several agents. Besides its health care assistance role, the SIAT has been participating in several studies about the potential environmental agents. The main objective of the present study is to analyze the type of consults and the assistance provided by the SIAT. Until June 2004, SIAT received 5,318 consults and 4,467 were analyzed. Physicians represented 42% of the total consultations. The general community presented 40%. There was an increase in the amount of inquiries from health care professionals since past years. With regards to the education level, 55.0% of non-health care individuals had concluded high-school or college. In terms of the main motif of the consultations, 71.0% were related to a specific pharmacologic substance, 12% related to other chemical substances and 4.7% related to radiation exposure and 4.0% maternal infections. The SIAT has an important assistance role for the health care professionals and for the general community while being a crucial source for better understanding the teratogenesis among humans.

Carrier Detection in Haemophilia using DNA Polymorphisms. *M. Singh¹, P. Singh¹, S.S. Mastana², K.P.S. Khushwaha³, H. Kaur⁴.* 1) Department of Human Biology, Punjabi University, Patiala, 147002, India; 2) Human Genetics Lab., Department of Human Sciences, Loughborough University, Loughborough, LE11 3TU, UK; 3) Forensic Science Lab., Madhuban, Karnal, Haryana, India; 4) St Boniface Hospital, Manitoba, Canada.

Background: The haemophilia is an X-linked recessive bleeding disorder associated with a chronic burden of morbidity and early mortality. It affects 1 in every 10,000 male births. Detection of carrier women and genetic counseling are important parts of haemophilia care and effective strategies for the genetic control of the disease. The aim of the present study was to assess the carrier status of the females in 52 families of the haemophiliac patients. **Methods :** Two polymorphisms of the factor VIII gene were analyzed using polymerase chain reaction, one on intron 18 recognized by BclI and another extragenic variable number tandem repeat (VNTR) locus recognized by St14. **Results :** There were 96 females and pedigree analysis revealed that 58 were probable carriers (whose carrier status was not known) and 38 obligatory (confirmed) carriers. Sixty one percent females were heterozygous for the intragenic polymorphism (BclI) whereas 86% females were informative for extragenic marker (St 14). Seven females were non informative with any of the marker used. Using both markers together, carrier status could be diagnosed in 92% of the females. **Conclusion:** Employing the above polymorphic markers of FVIII gene, diagnosis could be made in the majority of the families.

New genetic variability of flavin containing monooxygenase 3 and its functional characterization. *C. Charon*^{1,2}, *G. Steventon*¹, *C. Dolphin*¹. 1) King's College London, Pharmacy Department, UK; 2) CNG, Cell/DNA Department, Evry, France.

Flavin monooxygenase 3 (136132) is the principal human adult isoform of FMO expressed in the liver. A loss of function of the FMO3 gene on exon 4 was primarily found to be responsible for trimethylaminuria. FMO3 oxidizes sulfides into sulfoxides, phosphorous and nitrogen atoms in xenobiotics, environmental toxins and certain drugs such as cinetidine, ranitidine and antipsychotic medications. The most common polymorphisms and mutations coding for amino acids exchanged have already been reported (M66I, P153L, E158K, V257M, E305X, R492W, E308G). The discovery of SNP paves the way to the studies of inter-individual responses towards drug metabolism. DHPLC screening followed by sequencing revealed heterozygotes on positions c.341 (a>g), c.394 (g>c), c.441 (c>t), c.1055 (t>c), in a diverse ethnicity panel. Alleles frequencies were determined by PCR restriction digests in different cohorts. The amino acid change L352S was found in the African population. The non-synonymous variant N114S was found in both Chinese (6.6%) and Japanese (7%) populations whilst the polymorphism D132H was detected in Afro American (5%) and American Indians (10%). The synonymous biallelic marker S147 was present across all populations. Some individuals heterozygotes D132H were also heterozygotes at position 158. Among the polymorphisms cloned to determine the phase with the nearby common SNP the alleles were D132K158 and H132E158. FMO3 variants were produced after site directed mutagenesis in the heterologous baculovirus expression system (Bac-to-Bac, MOI of 5). Metimazole oxidation was affected by the microsomes isolated from cells infected with AcFMO3S114E158 and AcFMO3H132E158. Catalytic activity of human recombinant S114-FMO3 was greater than the human recombinant H132-FMO3, but at least 40% lower than the wt FMO3. The results indicate that polymorphisms found in non-caucasians could impair the oxidation of substrates. The substitution of aspartic acid into a basic amino acid histidine, H132, had a more drastic effect upon the protein activity than an asparagine-to-serine change at position 114. Both amino acids are essentials for FMO3 full enzyme activity.

Nonlinear Structural Equations for Reconstructing Genetic Networks. *L. Lin¹, X. Fang¹, M. Xiong²*. 1) Statistics, Beijing University, Beijing, China; 2) Human Genetics, University of Texas - Houston.

When the response of transcription of the gene to the activation of other genes and external stimuli is a nonlinear function of the expression levels of the activator genes, the genetic networks cannot be properly represented by a system of linear equations in both parameters and variables. In practice, gene regulations often show nonlinear relationship. Therefore, the general mathematical models for reconstructing genetic networks should incorporate nonlinear gene-gene interactions into the models. A general nonlinear Bayesian network model has not been fully developed and will not be easily developed even in the near future because its formulation is not very suited for developing nonlinear models. In this report, we propose to use nonlinear structural equations for modeling genetic networks. The full information maximum likelihood methods coupled by two extensions: (1) allowing random exogenous variables and (2) incorporating structure searching algorithms such as genetic algorithms and Tabu search into modeling development will be used to identify the structure of the genetic networks and estimate the parameters in the model. The proposed nonlinear structural equation model for genetic networks was applied to colon cancer data. The results shows that the nonlinear structural equations fit the data better than linear structural equations.

Discovery of novel disease genes by neural network approach to very large datasets. *M.A. Bakay¹, Z. Wang¹, O. McIntyre¹, J. Xyan², Y. Wang³, E.P. Hoffman¹.* 1) Center for Genetic Medicine, Children's National Medical Center, Washington, DC; 2) Computational Bioinformatics & Bioimaging Lab Dept of Electrical Engineering & Computer Science The Catholic University of America, Washington, DC; 3) Computational Bioinformatics & Bioimaging Laboratory Virginia Polytechnic Institute and State University, Alexandria, VA.

We hypothesized that diagnostic mRNA signatures could be developed by studying large numbers of mutation-known human muscle biopsies by mRNA expression profiling, using neural network classifiers, and then testing the performance of the system using bio-informatics methods.

We generated a 124 muscle biopsy data set (13 diagnostic groups) adhering to strict QC/SOP, then used a novel multilevel perceptron (MLP) approach for defining highly sensitive and specific diagnostic genes for each disease type. Each MLP at one node of the class grouping tree is responsible for classifying specific classes or groups, and the gene subset fed to each MLP is selected by focusing on the specific classes or groups involved in that classification task. We found high consistency between the diseases groupings and the biological background, suggesting pathway-specific information at the group-specific nodes.

We then tested a muscle biopsy of an undiagnosed muscular dystrophy patient and our Adaptive Hierarchical Subspace Explorer (AHSE) predicted that the unknown patient was affected with Calpain 3 (LGMD2A) disease. The Calpain 3 gene was sequenced and no mutation found. We then hypothesized that pathway-specific genes at the Calpain 3 node may be responsible for the patients disorder. We identified a single transcript whose behavior in the unknown is opposite to that in Calpain samples, suggesting nonsense mediated decay of this transcript. We sequenced this gene and found a 12 bp deletion that was not present in 186 control chromosomes. Sequencing of additional LGMD patients identified additional mutations.

Our data suggests that the Calpain 3 pathway-defined transcript is a new muscular dystrophy gene resulting in a form of limb-girdle muscular dystrophy.

High resolution microarray CGH of prostate cancer and application of custom informatics suite for analysis and annotation of genomic profiling. *B. Beheshti*¹, *S.K. Watson*², *W.L. Lam*², *J.A. Squire*¹. 1) University of Toronto, University Health Network, Toronto, Ontario, Canada; 2) BC Cancer Research Centre, Vancouver, British Columbia, Canada.

BACKGROUND: Molecular cytogenetic studies of early prostate cancers (CaP) and cell lines have shown frequent alterations on chromosomes 7, 8, 10, 13, 16, suggesting involvement of genomic intervals on these chromosomes in tumor development. Low resolution (> 10 MB) metaphase comparative genomic hybridization (CGH) fails to detect focal imbalances within cytobands that may harbor gene(s) of interest. High resolution microarray CGH offers fine structure mapping of genomic imbalance in these regions. **METHODS:** Genomic profiling of previously characterized CaP cell lines (LNCaP, DU145, PC3, 1532T, 1542T) was performed using Spectral Genomics BAC microarrays in order to delineate DNA dosage changes. Custom software (freely available at <http://www.utoronto.ca/cancyto/>) was produced in this laboratory to facilitate analysis of microarray CGH results, including data normalization, noise filtering, results plotting, LOWESS curve smoothing, & automated region imbalance determination. **RESULTS:** Genomic profiling refined and/or complemented existing characterization of CaP cell lines. For example, metaphase CGH of PC3 (Nupponen et al., 1998) identified two regions of gain at 1q21-q25 & 1q32-q44. No changes were detected at chromosome 3. Conversely, these same regions were refined by microarray CGH to be 1q23.3-q24.2 & 1q32.1-1q42.13, & novel gains were identified at 1pter-1p36.23 and 1q42.3-qter. The analysis also identified gains of 3p26.1-q21.2 (with interstitial deletion at 3p14.2-p14.1) and deletion of 3q22.1-q25.2. Other alterations will be presented. **DISCUSSION:** Our microarray CGH investigations, facilitated by custom analytical software, were able to refine an increased complexity of patterns of alterations in these cell lines than previously possible. These results are consistent with previous characterizations of prostate cancer, which have shown an ongoing genomic instability process perpetuating genomic imbalance and ploidy changes and contributing to the complexity of the karyotype observed by CGH.

Haplotype structure of human adrenergic receptor genes. *I. Belfer*^{1,2}, *B. Buzas*^{1,2}, *H. Hipp*^{1,2}, *G. Phillips*^{1,2}, *J. Taubman*², *I. Lorincz*^{1,2}, *C. Evans*^{1,2}, *R.H. Lipsky*², *M-A. Enoch*², *M.B. Max*¹, *D. Goldman*². 1) Pain and Neurosensory Mechanisms Branch, NIDCR, NIH, Department of Health and Human Services, Bethesda, MD; 2) Laboratory of Neurogenetics, NIAAA, NIH, Department of Health and Human Services, Rockville, MD.

Adrenergic receptors (AR) mediate the physiological effect of epinephrine and norepinephrine and are targets of a large class of therapeutic drugs. Interindividual differences in physiological states regulated by the adrenergic system may be in part due to genetic variations in the AR genes. We determined the haplotype architecture of nine human AR genes (*ADRA1A*, *ADRA1B*, *ADRA1D*, *ADRA2A*, *ADRA2B*, *ADRA2C*, *ADRB1*, *ADRB2* and *ADRB3*) in the Caucasian (CA) and African American (AA) populations. *ADRA1A* comprised of four haplotype blocks in CAs, while in the AAs Block 1 split. *ADRA1B* had four blocks in CAs, and in the AAs Block 4 split into two blocks. *ADRA1D* had 2 blocks in the CAs and Block 1 split into three blocks in the AAs. The *ADRA2A* and *ADRA2B* regions each had a single conserved haplotype block 11 kb and 16 kb in size, respectively, and spanning the entire gene, in both AAs and CAs. The *ADRA2C* gene had two haplotype blocks, 3 kb and 14 kb in size in CAs. In AAs Block 2 was split into two blocks 4 kb and 10 kb in size. For *ADRB1*, *ADRB2* and *ADRB3*, a single haplotype block was observed in both populations. In general we observed shorter haplotype blocks and more haplotype diversity in the AAs than in the CAs. The frequency of common haplotypes in each block differed significantly in the two populations. 3-5 common haplotypes were generally sufficient to give 80% population coverage, and a similar number of markers (2-5) per block was sufficient to capture maximum diversity in either population. For alpha 2 and beta AR genes, each marker panel included known functional markers. For each gene, haplotype captured the information content of each functional locus, even if that locus was not genotyped, and presumably haplotype would capture the signal from unknown functional loci whose alleles are of moderate abundance. Thus, these haplotype maps and marker panels are useful tools for genetic linkage studies.

UMD : a generic software to build Locus Specific DataBases. *C. Beroud¹, D. Hamroun¹, G. Collod-Beroud¹, S. Tuffery-Giraud¹, C. Boileau^{2, 3}, T. Soussi⁴, M. Claustres¹.* 1) Dept of Molecular Genetics, CHU de Montpellier, Montpellier, France; 2) INSERM U383, Paris, France; 3) Dept of Molecular Genetics, Boulogne, France; 4) Laboratoire de Genotoxicologie des tumeurs, Paris, France.

The creation of the Universal Mutation Database (UMD) tool was initiated 10 years ago and the first generic software was released in 2000. During this period the human genome project has released an almost complete sequence of the human. Thanks to the flexible database structure, the 2004 release of UMD includes all intronic and regulatory sequences of a specific gene. Other major developments have been performed to integrate clinical data, pictures, monoclonal antibodies and polymorphic markers. Since the beginning we focus on the creation of specific analysis tools. We have come a long way since the first application to the TP53 gene and the development of molecular epidemiology. Today UMD retains all specifically designed tools to analyze mutations at the molecular level and a new set of routines to search for genotype-phenotype correlations. With the creation of 25 LSDBs, almost 30.000 mutations are included in UMD-LSDBs. This led us to create specific tools for infrequent mutations such as gross deletions or duplications and deep intronic mutations. A set of dedicated routines is now available for intronic mutations (CV calculation of potential splice sites, search for ESE motifs). Other major developments have been performed to use graphical displays for various analyses such as geographic distribution of mutations. Concomitantly, we have created specific routines to help researchers to design new therapeutic strategies such as exon skipping, aminoglycosides read-through of stop codons or monoclonal antibodies selection and epitopes scanning for gene therapy. These tools pave the way for clinical trials and associations between genotypes and drugs response. The UMD generic software is today a reference tool to build LSDBs and is adapted to genes involved in genetic diseases or cancers. Since the beginning we have wrought to make a freely accessible tool of it. It can be downloaded at <http://www.umd.be>. We also offer to the various curators our hosting capacities for their UMD-LSDBs.

Ab-initio gene prediction in the minimum lost region at 17q21-23 and its partial characterization/annotation in sporadic breast cancer. *M. Bhuvanagiri*¹, *N. BAIRWA*², *R.N.K. BAMEZAI*¹. 1) NCAHG,SLS, Jawaharlal Nehru University+, New Delhi, India; 2) Department of Pathology,Carver College of Medicine, UIHC, University of Iowa, Iowa City, IA-52242.

The present study combined Insilco methodology with molecular biology approaches to map as yet un-annotated potential regions of putative tumor suppressor gene(s) in patients with sporadic breast cancer. Investigation of LOH in chromosomal region 17p13-3 covered by D17s5, D17s379 and 17q21.23 covered by D17s855, D17s934 and D17s787 showed maximum LOH in the regions surrounded by D17s934 and D17s787 markers in most of the tumor. Ab-initio gene prediction analysis of the genomic region surrounding D17S934 was carried out to search for putative TSG or novel gene(s). Insilco transcript map of the region was generated on Build 34 Sequence map information provided by NCBI. It was observed that approx 50kb region which included D17S934 marker showed sparse UNIGENE information. EST, Morbid and Breakpoint maps were studied using MAPVIEWER option provided by NCBI to know the EST's, disease association and breakpoints associated within the predicted region. Three genes were annotated in D17S787 and a gene with three exons was predicted in D17S934 region using GENSCAN. Further this region was analysed for in vitro presence of transcripts. Primers were designed using OLIGO software version 2.0, BLAST analysis was carried out to check the specificity of designed primers. The amplified genomic product was confirmed by sequencing using ABI PRISM 3100AVANT, Genetic Analyser. The experimental validation of one of the predicted exon revealed the presence of novel EST (Ac no-AY343912) in the D17S934 loci region. RT-PCR amplified product of these predicted exons was sequenced and when compared with genomic sequences using CLUSTALX software It turned out to be identical. The predicted coding regions were also analyzed for the presence of instability or somatic variation in tumor samples. This study exploits the sequence and map information generated by Human Genome Project, for finding novel expressed sequences that might be related to the sporadic form of breast cancer.

Program Nr: 1533 from the 2004 ASHG Annual Meeting

Speaking the Same Language. *E.A. Bruford^{1,2}, M.W. Wright^{1,2}, R.C. Lovering^{1,2}, M.J. Lush^{1,2}, H.M. Wain^{1,2}, V.K. Khodiyar^{1,2}, C.C. Talbot Jr.^{1,3}, S. Povey^{1,2}.* 1) HUGO Gene Nomenclature Committee (HGNC); 2) The Galton Laboratory, Department of Biology, University College London, Wolfson House, 4 Stephenson Way, London, NW1 2HE, UK; 3) The Johns Hopkins School of Medicine, Institute of Genetic Medicine, The Johns Hopkins University, Baltimore, MD, 21205, USA.

The aim of the HUGO Gene Nomenclature Committee (HGNC) is to designate unique and meaningful names and symbols to every human gene. Approved gene symbols are based on names describing structure, function or homology where possible. When placing genes in families we strongly encourage the development of hierarchical stem (or root) symbols, with a numbering system to distinguish individual members. This is an efficient and useful way to name large numbers of related genes. Some genes are not related by extensive homology or proven function, but rather by the presence of intrinsic domains and motifs. Members of such "gene groupings" might have an inferred function based upon these shared domains, and hence can be named on this basis. Examples of such families and groupings will be discussed. An exciting development in genome informatics is gene ontology (GO), which like the HGNC seeks to reduce confusion through the use of systematic controlled vocabularies. The HGNC is uniquely placed to provide GO terms for human genes as a result of the scientific evaluations, investigations and consultations undertaken during the nomenclature assignment process. Consequently, the HGNC are now working with the GO consortium to update their database. Assigning the same gene symbol to orthologous genes reduces interspecies confusion, and hence dedicated HUMOT (Human and Mouse Orthologous Annotation) curators have now been appointed at both the HGNC and the Mouse Genomic Nomenclature Committee (MGNC). These curators will liaise on human/mouse orthology problems to enhance communication and further strengthen collaboration between the two committees. Illustrations of the effects of these developments with GO and the MGNC on human gene nomenclature will be provided. Please come and visit us at our booth in the Exhibit Area, or go to <http://www.gene.ucl.ac.uk/nomenclature/> for further information. Supported by NIH contract N01-LM-9-3533.

Comparative genomic approach to disease gene discovery: Application to Bardet-Biedl Syndrome (BBS3). *A.P. Chiang¹, D.Y. Nishimura¹, K. Elbedour³, R. Carmi³, T.E. Scheetz¹, T.A. Braun¹, E.M. Stone^{1,2}, T.L. Casavant¹, V.C. Sheffield^{1,2}.* 1) University of Iowa, Iowa City, IA; 2) Howard Hughes Medical Institute, University of Iowa, Iowa City, IA; 3) Genetic Institute, Soroka Medical Center, Ben Gurion University of the Negev, Beer-Sheva, Israel.

We report a computational comparative genomics strategy that identifies biological features shared by some organisms, but absent in other species, and utilizes this information for the identification and ranking of disease candidate genes. We successfully apply this approach to identify a Bardet Biedl Syndrome (BBS3) gene. BBS is a genetically heterogeneous disorder characterized by obesity, retinal degeneration, polydactyly, male hypogonadism, renal abnormalities and cognitive impairment. One of the eight known BBS loci (BBS3) was previously mapped to chromosome 3 by using linkage analysis of a single large inbred Israeli Bedouin kindred. We refined the BBS3 locus to a region of ~5 cM. A paucity of other BBS3 families limited further refinement of the disease interval. We hypothesized that the genomes of model organisms that contain the orthologs to known BBS genes would also likely contain BBS3 orthologs. We implemented a computational approach that prioritized genes in the ~5 cM BBS3 genetic interval based upon conservation in the genomes of species that have orthologs of the previously known BBS genes. Subsequent sequencing of prioritized candidate genes led to the identification of a homozygous stop mutation in an ADP-ribosylation factor-like (ARL) gene in all affected members of the original BBS3 pedigree. We hypothesize that a generalization of this comparative genomic approach may be employed in the study of other genetic disorders.

Program Nr: 1535 from the 2004 ASHG Annual Meeting

CodeLink Whole Genome High Performance Bioarrays:For Comprehensive Gene Expression Profiling. *B. Chui, G.L. Kiser, T. Giesler, R. Svirskas, P. Gwynne, M.E. Morrison, J. McGowen, R. Lockner, R. Bonner, M. Gaskin, R. Shippy, T. Sendera, H. Samartzidou, T. Kaysser-Kranich.* CodeLink, GE Healthcare, Chandler, AZ.

CodeLink Whole Genome Bioarray product offerings, including the CodeLink Human and Rat Whole Genome Bioarrays, are the result of three main technological improvements: a new slide surface, production robotics for dispensing, and software for spot quantification and data analysis. These improvements enabled increased density and performance leading to bioarrays with the most comprehensive genome coverage for human and rat gene expression profiling. These bioarrays show typical CodeLink high reproducibility and offer sensitivity down to 1:1,024,00 (estimated mass ratio), with a linear dynamic range of ~3 logs. In addition to the high density bioarrays, the system includes optimized reagents and protocols for target preparation, hybridization, secondary-labelling, and detection, as well as software for data extraction and analysis. Content is based on the UniGene, RefSeq and Ensembl public databases. An improved control probe set includes 384 negative control and 360 positive control bacterial sequence probes. The Rat Whole Genome Bioarray System provides expression profiling of ~35,000 rat gene targets in a single bioarray. Here we describe the performance of this Whole Genome Bioarray System.

Divergent evolution of GAA triplet repeats in primate genomes. *R.M. Clark, S.S. Bhaskar, M. Miyahara, S.I. Bidichandani.* Biochemistry & Molecular Biology, University of Oklahoma Health Sci Ctr, Oklahoma City, OK.

Abnormal expansion of triplet repeats causes several neurodegenerative diseases. While CAG/CTG and CGG/CCG expansions cause several disease phenotypes, expansion of GAA/TTC repeats is presently only known to cause Friedreich ataxia. We have previously shown that of all ten triplet repeats, GAA repeats have expanded the most in the human genome. There are 29 instances of (GAA)₃₀₊ sequences in the draft human genome, i.e. corresponding to premutation alleles that display germline instability at the Friedreich ataxia locus. Given our previous observation that GAA triplet repeats overlap with Alu elements, we were curious whether these potentially premutagenic GAA triplet repeats also exist in non-human primates. Analysis of all ten triplet repeats in the entire chimpanzee genome also revealed that GAA triplet repeats have undergone the most expansion, with 56 instances of (GAA)₂₅₊ sequences. However, the loci containing long GAA repeats in the human genome did not correspond to those in the chimpanzee genome, and vice versa. Indeed, in 70% of cases the homologous chimpanzee loci of the human (GAA)₃₀₊ sequences contained <8 repeats. Likewise, 45% of human loci corresponding to the chimpanzee loci with (GAA)₂₅₊ had <8 repeats. In two cases we even found TAA repeats at the corresponding human loci in place of (GAA)₂₅₊ in the chimpanzee genome. To evaluate the validity of the potentially unstable GAA sequences we analyzed 50-100 normal human chromosomes at nine of the human (GAA)₃₀₊ loci (at which the chimpanzee genome has <8 triplets). At seven of these nine loci, chromosomes were identified that carried >100 GAA triplet repeats, corresponding to disease-causing expansions at the Friedreich ataxia locus. Our data indicate that while GAA triplet repeats have undergone significant expansion in primate genomes, individual loci show substantial inter-species divergence. These differences constitute a potential source of difference at the genomic level between humans and chimpanzees, species that otherwise share >95% sequence homology.

GeneRetriever extracts the data needed to design a custom microarray containing probes of all genes and transcripts in between two genetic markers. *M. Clement-Ziza¹, Y. Brody², A. Munnich¹, S. Lyonnet¹, C. Besmond¹.* 1) INSERM U393, Hopital Necker-Enfants Malades, Paris, France; 2) Department of Biology and Computer Science, Bar Ilan University, Ramat Gan, Israel.

Identifying the genes that are responsible for human genetic disorders with complex inheritance patterns (e.g. multigenic diseases) proves to be somehow difficult. Indeed, as the mode of inheritance is unknown, classical parametric linkage studies are not relevant. Nonparametric linkage analysis can help approximate the candidate loci. However, this type of analysis often leads to the identification of large intervals (10-20 cM) which may contain hundreds of genes thus rendering the candidate gene approach rather tedious. Microarray expression studies of the genes located within such genetic intervals and performed on relevant tissues may significantly reduce the number of candidate genes. The design of a custom microarray containing probes of all the genes located in the intervals of interest is required to carry out such experiments. Designing such microarrays involves gathering much additional information concerning these genes. Collecting these data manually is time-consuming and very error-prone.

GeneRetriever is a Perl-based data mining tool developed to automate, accelerate, and secure the process of retrieving, from NCBI databases, user chosen information needed to design a custom expression microarray containing all genes and transcripts located in between two genetic markers. These data include gene description (full gene name), DNA strand, type of gene (either known or predicted), cytogenetic localization, summary of functional annotations, Entrez Gene identifier, as well as the number of transcripts for each gene. Versioned GeneBank accession numbers, structural information (i.e. number of exons) and nucleotide sequences of transcripts can also be collected. GeneRetriever provides both graphical and command-line interfaces. It is fast and requires only an Internet connection. GeneRetriever was used to help design a custom microarray with all genes from three genetic intervals involved in Hirschsprung disease.

Program Nr: 1538 from the 2004 ASHG Annual Meeting

Annotation tools for human and mouse Affymetrix gene expression microarrays. *R.J. Clifford, K.H. Buetow.*
National Cancer Inst, Bethesda, MD.

The Affymetrix GeneChip microarray is a popular platform for gene expression studies. GeneChips were designed against clusters from past UniGene clusters builds: the HG-U133 series, for example, is based on clusters from UniGene build Hs133. Since the design of these arrays, UniGene clusters have been refined in response to improved knowledge of gene structure. Clusters containing sequences from homologous genes have been split. Distinct UniGenes representing alternative products of a single gene arising from differential splicing or polyadenylation site selection have been merged. Unnamed genes have been given HUGO approved gene symbols and novel loci have been discovered. For these reasons it is critical to maintain current annotations for GeneChip probe sets. To address this need, Affymetrix provides quarterly annotation updates on its website. We provide an alternative set of annotations for the human HG-U95 and HG-U133 and murine MG-U74 and MOE430 chip sets. At each UniGene update, we use BLAST to map each microarray probe sequence against the complete set of mRNA and ESTs sequences included in the current UniGene build. Our WWW-based tool (<http://lpgws.nci.nih.gov/cgi-bin/AffyViewer.cgi>) displays human and mouse GeneChip probes in the context of mRNA sequences. Antisense probes and probes that do not specifically recognize their target loci are flagged. The BLAST evidence used to map probes onto transcripts and genes is also available. Plain text annotation files and mask files can be downloaded via FTP. This work utilizes the high-performance computational capabilities of the Biowulf PC/Linux cluster at the National Institutes of Health, Bethesda, MD (<http://biowulf.nih.gov>).

Identification of Xenobiotic-responsive Target genes by Tandem Machine Learning. *D. Dinakarbandian¹, V. Raheja¹, S. Mehta¹, P.K. Rogan^{1, 2}.* 1) Schools of Computing and Engineering and; 2) Medicine, Children's Mercy Hospital, University of Missouri-Kansas City, MO.

Motivation: One of the challenges in Bioinformatics is to understand the regulatory signals embedded within the human genome that are responsible for the initiation and regulation of DNA transcription.

Approach: In this collaborative project, we have developed a tandem machine learning approach to the identification of target genes for the xenobiotic-sensitive transcription factor PXR/RXR within the human genome. An information theory-based weight matrix was first derived and refined from validated PXR/RXR binding sites. Then, gene candidates identified by microarray and published studies that were known to be regulated by this transcription factor were scanned to identify putative binding sites. An information density-based clustering algorithm was then used to identify clusters of information rich sites. Finally, transformed data representing metrics of location, strength and clustering of binding sites were used for classification of promoter regions using an ensemble approach involving neural networks, decision trees and Naive Bayesian classification.

Results: The method was evaluated on a set of 24 known target genes and 288 genes known not to be regulated by PXR/RXR. We report an average accuracy (proportion of correctly classified promoter regions) of 71%, sensitivity of 73%, and specificity of 70% based on n-way cross-validation and exhaustive leave-out-one strategy. The performance on an unknown test set of 10 genes showed that 8 (80%) were correctly classified.

Conclusion: We find that the clustering of sites with high individual information contents is an important determinant of target specificity. While the goal is to identify all genes that are regulated by PXR/RXR, the method is general enough to be applicable to the identification of target genes for other transcription factors..

GeneSniffer - a gene prioritisation tool. *K.S. Elliott^{1,2}, J. Blangero^{2,3}, J. Jowett^{1,2}*. 1) International Diabetes Institute, Australia; 2) Chemgenix Pharmaceuticals, Australia; 3) Southwest Foundation for Biomedical Research, USA.

Localization of genes influencing susceptibility to complex diseases generally results in the need to evaluate numerous potential candidates in a large genomic interval. The choice of positional candidate genes is usually based on subjective criteria and the invocation of luck. In this presentation, we describe a semi-quantitative objective method for the efficient prioritisation of positional candidate genes in regions of linkage. The strategy involves computer-intensive database mining for bioinformation on the genes under a linkage peak as a prelude to the expensive molecular lab work subsequently required. This process can be tedious and very time consuming since there can be hundreds of genes to choose from for further analysis. GeneSniffer is a computer program developed in house to assist with this task. For each gene within a given linkage interval, GeneSniffer downloads appropriate webpages from the Locuslink, OMIM and PubMed databases and interrogates the text using a list of disease specific keywords (assigned a score between 1-10 depending on significance). Homologs of each gene are identified by BLAST and scored for content of their Locuslink, OMIM and PubMed entries. These scores are weighted according to the degree of homology and a cumulative hit-score is calculated for each gene. Jackson pages for available mouse orthologs are also scored. The resultant output is presented in linked webpages (in html format) to provide the investigator with information as to the source of the hits and links to relevant external webpages for further information. The method leads to an objective ranking of genes based on multiple criteria that can be further processed statistically and graphically if desired. Like all bioinformatic methods based on potentially error-prone public databases, the output of the program still needs to be closely examined, but significant time is saved compared to manually sifting through the large quantity of data. Additionally, the process takes advantage of the dynamic nature of public databases since scripts can be rerun at any time to incorporate database updates.

Subspace Identification for Genetic Networks. *X. Fang*¹, *L. Jin*³, *M. Xiong*². 1) Dept Statistics, Beijing Univ, Beijing; 2) Human Genetics, University of Texas - Houston; 3) University of Cincinnati.

Genetic networks are complex systems that require accomplishing complex tasks with high accuracy. A very powerful approach to modeling complex systems is the state-space approach. The state-space approach is a good choice for modeling regulation due to the following reasons. First, the concept of state variables is very suitable for description of the regulatory process. Second, the state-space approach is in the core of modern control theory. Third, development of RNA interference for repression of gene expression and synthetic biology in designing artificial regulatory circuits. Their successful applications to the development of smart drugs require state-space representation of dynamics of genetic networks and applications of modern control theory to design and analysis of genetic networks. State-space approach provides a general framework and a powerful tool for application of control theory to analysis of genetic networks. A key issue for modeling genetic networks is how to model regulation of a gene. We first use state space equations with two state variables for modeling a transcriptional process of the gene. We then use block diagram as a graphical representation of the genetic networks. We define mathematical operations on the block diagram to organize genes into a genetic network. The parameters in the genetic networks are estimated by subspace method, a new generation of system identification algorithms. The proposed model and estimation procedures were applied to DNA Repair network in *E. Coli*. The accuracy of the estimation by the state-space model is even higher than that of kinetic models.

The Mutation Database Initiative 10 years on: where are we today? *O. Horaitis*¹, *R.G.H. Cotton*^{1, 2}. 1) Genomic Disorders Research Ctr, Melbourne, Australia; 2) The Univ. of Melbourne, Dept of Medicine, Australia.

Ten years ago at ASHG Montreal, a small meeting was organized to discuss the problem of incomplete mutation collection with a group of the worlds senior geneticists. Issues such as non-uniform nomenclature of mutations throughout publications and databases, databases being mutation focussed rather than proband/phenotype focussed and central database curators not being experts in specific genes were discussed. From this meeting stemmed a group known as the Mutation Database Initiative that quickly grew to around 600 members interested in capturing human genome variation. In October 2001 at the annual meeting at ASHG it was decided that the group should become a formal Society and it is now known as the Human Genome Variation Society (HGVS; www.hgvs.org), of which there are almost 100 members to date and growing. The main objectives of the HGVS remain to ensure documentation, collection and free distribution of all variation information. We have made progress in areas of standardization of mutation nomenclature- our nomenclature is now accepted as the standard in naming variations, the creation of Locus Specific DataBases for a large number of genes- 85 new gene-specific databases have been created due to our encouragement, the creation of databases specific to ethnic or national groups- there are now databases specific for Arab, Cypriot, Finnish, Greek, Iranian and Turkish genetic disease with others under construction. Recommendations for quality control of data and standardizing database content in mutation databases have been made and many databases comply. The design of an Allele Variant Entry Form to be used for submission of data to LSDBs is now being used by 24% of LSDBs that allow submission of mutations directly to their database for their collection of mutation data. A comprehensive alphabetically organised by gene catalogue of LSDBs was created and is maintained in online format. Some mutations discovered in the research or diagnostic laboratory will never be reported. The challenge is to catch these mutations and disseminate them through the WWW for access by all; this is the raison d'etre of the MDI now HGVS. See www.hgvs.org.

Comparison of evaluation methods for the quality control of diagnostic oligonucleotide chip. *C.M. Kim^{1, 2}, H.K. Park², H.J. Jang³*. 1) Department of Biochemistry, College of Medicine, Pusan Natl Univ, Busan, Korea; 2) Busan Genome Center, College of Medicine, Pusan Natl Univ, Busan, Korea; 3) Department of Microbiology, College of Natural Science, Pusan Natl Univ, Busan, Korea.

The monitoring of spot-uniformity and probe-attachment and quantification of mixed type of target product are critical to manufacturing the diagnostic oligonucleotide chip. In this study, we described a new quality control (QC) probes as a means of monitoring uniformity of attached probes and quantifying mixed types of target products. We compared the results of the evaluation methods containing QC and target probes and methods containing only target probe of oligonucleotide chip. We designed two types of QC probes. The T20-F probe was comprised 20-mer poly T and was labeled by the TAMRA dye at the 3end. The T20-F probe was mixed with target specific probes for oligonucleotide chip fabrication. The END-F probe was labeled by the TAMRA dye at the 3end of target specific probe. The END-F probe was performed at once QC probe and target specific probe. The fluorescent images by a QC and target probes were obtained at 532 nm and 635 nm. The oligonucleotide chip containing T20-F or END-F probe could be assessed spot quality of all process. But the oligonucleotide chip containing only target probe could not be assessed spot quality. To quantify mixed type of target product, we used signal intensity of QC probe to normalize variations in spot quality and hybridization. The two biotin labeled-PCR products were mixed at 0:10, 1:9, 5:5, 9:1, and 10:0 wt-to-mutant ratios for each concentration. The oligonucleotide chip contained QC probe was hybridized with biotin-labeled target product. We could distinguish ratio of major and minor from mixed type. Two QC probes could detect from 0:10 to 10:0 of that compared to signal intensity of QC probe and target probe (in case of T20-F probe, 1:5.5 at 0:10, 1:2.2 at 1:9, 1:1.1 at 5:5, 1:0.2 at 9:1, and 1:0.1 at 10:0). However, the oligonucleotide chip containing only the target probe could not distinguish ratio. This methods containing quality control probe can assess spot quality of all process and quantify ratio of major and minor from mixed type.

Identification of 93 hair keratin-associated protein (KAP) genes in human genome. *J. Kudoh¹, K. Shibuya¹, I. Obayashi¹, S. Yahagi^{1, 2}, T. Sasaki¹, A. Shimizu¹, N. Shimizu¹.* 1) Dept Molecular Biology, Keio Univ Sch Medicine, Tokyo, Japan; 2) COSMOS Technical Center Co., Ltd., Tokyo, Japan.

Major components of human hair are keratin intermediate filaments (KIFs) and keratin associated proteins (KAPs). KAPs are low-molecular weight proteins and have been classified into four types according to their amino acid compositions; ultra-high sulfur (UHS) KAP (>30% for cysteine content), high sulfur (HS) KAP (16-30% for cysteine content), high glycine-tyrosine (HGT) KAP (35-60% for glycine and tyrosine content), and the unconventional type which belongs to neither UHS/HS nor HGT type. KAP genes (gene symbol KRTAP) consist of single exon and can hardly be identified by exon prediction software. We analyzed human whole genome sequence and found that 93 KAP genes are located as 5 separate clusters (11p15.5, 11q13.5, 17q12-q21, 21q22.11, and 21q22.3) in the human genome. The cluster on 11p15.5 spans over 110 kb and consists of 6 authentic genes for UHS KAP. The cluster on 11q13.5 spans over 56 kb and consists of 5 authentic genes and 2 pseudogenes for UHS KAP. Precise analysis of the cluster on 17q12-q21 revealed that the cluster consists of 34 authentic genes for HS or UHS KAPs. The cluster on 21q22.11 spans over 800 kb and consists of 32 authentic genes and 18 pseudogenes for HGT KAPs or the unconventional type KAPs. Eight out of 32 authentic genes are newly identified genes including 4 novel types of KAP genes for the unconventional type KAPs. The cluster on 21q22.3 spans over 165 kb and consists of 16 authentic genes and 5 pseudogenes for HS KAP. Interestingly, all these authentic genes are located within introns of another gene TSPEAR (gene symbol C21orf29) spanning over 214 kb. Surprisingly, transcriptional direction of 8 out of 16 authentic genes is the same as that of TSPEAR gene. This finding suggests a novel transcription mechanism in which TSPEAR gene transcription passes over the multiple transcriptional termination sites of the KAP genes. Furthermore, we have identified similar KAP gene clusters in two primates, chimpanzee and baboon, and the detailed comparison revealed the process of KAP gene cluster formation during evolution.

Intra-species genome comparison reveals 802 polymorphic Alus and dynamic Alu distributions in human

genome. *P. Liang*¹, *J. Wang*¹, *L. Song*¹, *M.K. Gonder*², *S.A. Tishkoff*², *S. Azrak*¹. 1) Cancer Genetics, Roswell Park Cancer Institute, Buffalo, NY; 2) Biology, University of Maryland, College Park, MD.

Alu elements, as the most active and successful SINE-type retrotransposons constituting 10% of the human genome, are now known to play important roles in human genome. By employing an in silico approach to compare the public and Celera versions of human genome sequences, we identified a total of 802 polymorphic Alu elements with over 750 being novel. PCR screening of a randomly selected 16 Alus using a panel of diversified human DNA samples revealed a very high specificity of the method. Our unbiased analysis of all Alu subfamilies reveals that Alu insertion activity differs dramatically across Alu subfamilies with Ya5, Yb8, Yb9, and Ya4b being the most active and a few S subfamilies also showing a low level of activity. These polymorphic Alus, representing the most recent Alu insertions in human genomes, are characterized by the presence of target site duplications and longer polyA-tails with the pre-integration sites mostly following the NT-AARA motif. Like all Alus in general, these recent Alus are distributed heterogeneously in the genome. Very interestingly, the density of old Alus in chromosomes is positively correlated to the gene density accumulating against the availability of Alu sites. In contrast, the density of new Alus is reversibly correlated with Alu and gene density, but positively correlated with Alu site availability. As an exception, Y chromosome has the lowest density of both old and new Alus, although it has an average level of Alu site availability and gene density, suggesting the existence of a mechanism suppressing Alu insertions in Y chromosome. While old Alus show accumulation towards the gene-rich regions, particularly the promoter regions, the new Alus are biased towards intergenic regions, suggesting that a post-insertion selection mechanism is responsible for the biased accumulation of old Alus in gene regions. Our report presents the first attempt to identify all polymorphic Alus between two individuals, and the unprecedented large list of polymorphic Alu markers offers new opportunities for comprehensive population genetics studies.

MutScreener: a web-based tool of primer design for mutation screening by PCR-direct sequencing. *C. Liu^{1,2}, F. Yao², R. Zhang², Z. Zhu², J. Xia², E.S. Gershon¹*. 1) Dept Psychiatry, Knapp Res Ctr, Univ Chicago, Chicago, IL; 2) National Lab of Medical Genetics of China, Central South University, Changsha, P.R. China.

Polymerase Chain Reaction (PCR) amplification with direct DNA sequencing is the major technology for mutation screening of candidate genes. Optimal PCR primer design is one of the most important procedures to obtain high quality results. In order to perform automated annotation of human candidate genes and PCR primer design for mutation screening, we developed a web application named MutScreener. BLAT, Primer3 and DNannotator are used as major data processing components. MutScreener uses sequence data from NCBI and UCSC genome databases or from users own lab. It has three different tracks to handle three different types of input data: 1) cDNA sequence or Genbank ID and human chromosome number; 2) gene annotation produced by DNannotator and users own genomic DNA sequence; 3) gene annotation from Genome Browser. It can analyze genes intron/exon structure (if the first track is used) and pick up multiple PCR and DNA sequencing primers for multiple exons of multiple genes. The second track gives the user opportunity to re-sequence target regions other than promoters and exons, as the annotation features table can be edited. Splicing sites and low quality sequencing region produced in sequencing machine are considered to ensure complete coverage of high quality sequencing data over target sequences. Results are in tabular text format for easy management. MutScreener can be accessed at <http://sky.bsd.uchicago.edu/MutScreener.html>.

Prediction of TaqMan SNP assay genotyping performance of Whole Genome Amplified DNA using wgaDNA yield and STR genotyping performance. *Y. Qi¹, A. Bergen^{1,2}, K. Haque¹, M. Beerman¹, R. Welch¹, S. Chanock^{1,2}.* 1) Core Genotyping Facility, National Cancer Institute, 8717 Grovemont Circle, Bethesda, MD 20877; 2) Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Boulevard, MSC 7242, Bethesda, Maryland 20892-7335.

The aim of this study was to identify predictors of SNP genotyping performance. Data were collected from an evaluation of Whole Genome Amplification (WGA) using genomic DNA (gDNA) derived from lymphoblastoid cell lines and using a range of gDNA inputs (1-100ng). gDNA from 22 lymphoblastoid cell lines was amplified using a commercially available Multiple Displacement Amplification WGA protocol. Whole Genome Amplified DNA (wgaDNA) was quantified by UV spectroscopy, the PicoGreen assay (Molecular Probes), and a quantitative TaqMan assay specific to human DNA. wgaDNA genotyping performance was evaluated using genotypes determined from both gDNA and wgaDNA using the N=15 STRs in the AmpFISTR Identifiler assay and N=49 TaqMan SNP genotyping assays. We observed a significant positive correlation ($p < 0.01$) between STR and SNP genotyping performance. Linear regression analyses were performed to determine the wgaDNA yield and AmpFISTR Identifiler STR genotyping performance factors that are significantly associated with wgaDNA TaqMan SNP genotyping assay performance. Two models were constructed with dependent variables SNP completed genotypes and SNP concordant genotypes, respectively. Independent variables included: STR completed genotypes, STR concordant genotypes, STR Genotype Quality score, STR Peak height (Allele 2), wgaDNA yield (as measured by quantitative PCR), and gDNA input into WGA reaction. In the model, SNP completed/concordant genotypes are the total number of completed/concordant genotypes over 49 SNP assays and STR completed/concordant genotypes are the total number of completed/concordant genotypes over 15 STR assays. The final best predictive model included STR completed genotypes and STR concordant genotypes, with a R-Square of 0.9987 and p values 0.0001. Completed genotypes from 15 STR markers can be used to predict completed genotypes from 49 SNP assays with a linear regression model.

Large-scale Production of BAC Clone Representations for Microarray Comparative Genome Hybridization. D. Smailus¹, M. Krzywinski¹, J. Stott¹, C. Mathewson¹, D. Lee¹, P.J. de Jong², D. Albertson³, J. Friedman⁴, M. Marra¹, J. Schein¹, R. Holt¹. 1) BC Cancer Agency , Genome Sciences Centre, Vancouver, BC Canada; 2) BACPAC Resources, Children's Hospital Oakland Research Institute, Oakland, CA USA; 3) UCSF Cancer Center, San Francisco, CA USA; 4) Dept. of Medical Genetics, University of BC, Vancouver, BC CANADA.

Microarray comparative genome hybridization (maCGH) detects chromosomal aberrations that result in DNA copy number changes. Bacterial artificial chromosome (BAC) clones are commonly used as hybridization targets for maCGH. The large insert size of BACs allows integration of hybridization signal over a large region and provides sufficient sensitivity to routinely detect single copy number changes. Previously, we selected a tiling set of 32,855 human BACs from various libraries that gives 75 kb average sampling resolution and 99% coverage of the sequenced portion of the genome (Krzywinski et al., *Nucleic Acids Res.*, in press). These clones have been arrayed into chromosome specific sets and validated by restriction fingerprinting. Because preparation of BAC DNA is labour intensive and gives low yields of high molecular weight (and therefore difficult to handle) DNA, we have implemented an automated, high-throughput ligation-mediated PCR pipeline to generate BAC clone representations suitable for spotting. Briefly, clone DNA is prepared by alkaline lysis, digested with MseI, and oligonucleotide adapters are ligated to the ends of fragments. The fragments are amplified by two rounds of PCR using adapter-specific primers. Final PCR products (~10ug per clone) are precipitated, then re-suspended overnight in 15 ul of 20% DMSO solution. A Beckman Coulter BiomekFX dual head 96-tip P20/200 liquid handler with ART barrier tips is used for all liquid handling steps. Using this method we are currently generating approximately 6000 BAC clone representations per week, for use in fabrication of a whole genome tiling microarray.

The SPEED Toolkit: A resource for evolutionary analysis in Human genetic studies. *A. Solidar*¹, *J.E. Paschall*¹, *C.M. Malcom*², *G.J. Wyckoff*¹. 1) Div. Mol. Biol. and Biochem, U. Missouri-Kansas City, Kansas City, MO; 2) 2Dept. of Anthropology, The University of Chicago, Chicago, IL.

We detail a website devoted to integrating evolutionary information into human genetic studies. This suite of tools is geared toward choosing genes of clinical interest, targets for genomic or proteomic analysis, and possible human disease genes. The database allows for evolutionary analysis of genes that have been under-studied because their adaptive significance has been underestimated. This is particularly important in light of recent data that suggest that evolutionary rate and constraint may be correlated with the likelihood that a gene is involved with human disease. The website, located at <http://bioinfobase.umkc.edu/>, is open to the public, and all information within is annotated so that even scientists with little formal evolutionary training can grasp the meaning and utility of the information presented. The website itself is carefully designed to allow queries from a wide variety of starting perspectives. For example, the toolkit includes a search for disease association, a user-defined protein motif search, and a browsing tool for relative evolutionary rate. As an example of the toolkits utility, we show how a researcher using a peptide sequence, gathered for example from an MS/MS experiment, can query the database to obtain a set of genes, with evolutionary information, within a few minutes. These techniques save enormous amounts of analysis time and can be integrated into a pipeline for current laboratory analysis procedures. The rational, multi-entry design of the website means that obtaining interesting and useful results is quick and can be performed by investigators at multiple levels: from simple, exploratory assays to thorough, detailed analyses for publication.

Accurate detection and classification of heterozygous indels by direct sequencing. *J. Sorenson, A. Pradhan, S. Vijaychander, B. Sangari, T. Nguyen, B. Jones, D. Guo, Q. Doan.* Applied Biosystems, Foster City, CA 94404.

A heterozygous insertion/deletion (indel) mutation is defined by the presence of two alleles differing by an insertion or deletion---a length polymorphism. It has been estimated that 20% of the polymorphisms in the human genome are length polymorphisms. However the direct sequencing of heterozygous individuals is complicated by a phase shift in the electropherogram trace at the site of the indel mutation. Quality-based sequencing pipelines discard this data as noise or spend significant time and effort trying to call the polymorphism correctly. Starting with SeqScape Software v2.0, we introduced an algorithm to predict when samples were exhibiting the presence of a heterozygous indel mutation (HIM). This algorithm has been refined in subsequent releases of the software to include basecalling the inserted/deleted sequence, mapping the polymorphism to the reference sequence, displaying the mutation in the HUGO-approved format, and assigning a quality value to the mutation. Recently we have been able to improve the algorithm further by accumulating numerous in-house and customer examples of this trace feature and developing a pipeline for annotation and automated testing. Results from this testing show a significant improvement in the ability of the software to correctly call heterozygous indel mutations versus earlier analysis pipelines. This ability to accurately detect and characterize heterozygous indels offers a valuable solution to a previously difficult problem in the analysis of resequencing data.

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Visualization of valid polymorphisms within the human genome. *B.J. Staats¹, L. Qi¹, M. Beerman¹, H. Sicotte¹, B.R. Packer¹, S.J. Chanock², M. Yeager¹.* 1) Intramural Research Support Program, SAIC-Frederick, NCI-FCRDC, Frederick, MD, USA; 2) Section on Genomic Variation, Pediatric Oncology Branch, National Cancer Institute, National Institutes of Health, Gaithersburg, MD, USA.

The Annotator web application serves as a tool for viewing publicly available variations together with those validated by the Core Genotyping Facility (CGF) at the National Cancer Institute. Users are able to see human variation in genomic and proteomic contexts along with population-specific information, enabling them to make informative decisions based on the polymorphisms that are present in a given population. Currently, users can search by gene or variation to retrieve a gene-centric view of the gene and/ or variation. Intergenic regions are also viewable. CGF administrators can add new annotations from the CGFs SNP discovery/validation pipeline directly through the Annotator user interface in an intuitive and productive manner. The Annotator is linked to other CGF databases such as the laboratory information management system, to automate annotations relevant for assay design and validation. Among the many links the Annotator has to other resources, there are links to and from the SNP500 cancer web site which presents data generated by the CGF. Ongoing development for the Annotator includes visual analysis results from genotype data and additional information from public resources.

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SNPHunter: A Bioinformatic Software for Single Nucleotide Polymorphism Data Acquisition and Management.

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SNPs serve as important markers for identifying genetic variants for human diseases. Current public database (dbSNP) contains information for ~9 millions SNPs in the human genome, half of which with varying degree of confirmation. Retrieval of SNP data from dbSNP has emerged as a major bottleneck in genetic research. We developed a software tool, SNPHunter, which retrieves SNP data (e.g. physical map position, function class, flanking sequences, heterozygosity, and confirmation status) from NCBI dbSNP website according to user specified criteria, and allows downloading of such data for local storage and management. SNPHunter consists of three functional modules: (1) Direct dbSNP Query ("SNPSearch"); (2) SNP Data Extraction and Management ("SNP Management"); and (3) LocusLink SNP Query ("LocusLink SNP"). In these modules, SNPHunter retrieves SNPs according to gene list and automatically filter SNPs by interacting with user, which greatly facilitate candidate SNP selection in genetic studies. Furthermore, SNPHunter can extract SNP flanking sequence with annotation of all SNPs within the sequence. As an illustration, we applied SNPHunter for selecting SNPs for 10 major candidate genes for type 2 diabetes. SNPHunter does not require any local database support, and always retrieve the latest SNP and sequence data from NCBI. Its user-friendly design offers researcher an easy and intuitive interface and it is freely available at:<http://www.hsph.harvard.edu/ppg/software.htm>.

Global landscape of unusual genetic architectures for *Homo sapiens*. E. Wang^{1,3}, G. Kodama², P. Baldi^{1,2,3}, R. Moyzis^{1,3}. 1) Biological Chemistry, Univ of California, Irvine, Irvine, CA; 2) School of Information and Computer Science, University of California, Irvine. Irvine, CA 92697; 3) Institute of Genomics and Bioinformatics, University of California, Irvine, Irvine, CA 92697.

From our previous reports of the unusual genetic architecture of the human Dopamine Receptor D4 (*DRD4*) gene locus, we noted that a Bayesian statistical model could be used to describe the signature left by directional selection. With the availability of the International Human Haplotype Map (HapMap) data, we use this approach to uncover the global landscape of unusual genetic architectures associated with recent selection. We ask how often would we find regions similar to known selected genes such as the human Glucose-6-phosphate Dehydrogenase (*G6PD*) V202M locus? Our model describes a scenario where complete Linkage Disequilibrium (LD) for one allele does not decay to noise until 500,000 base pairs away. Here, we show that such inferred selective events are not random and quite common in human DNA. Following literature-based confirmation, we outline the major statistically over-represented Gene Ontology (GO) categories, representing several predominant biological themes. These over-represented categories include various systems likely associated with pathogen response, genes associated with DNA metabolism/cell cycle/gametogenesis, and pathways associated with neuronal function.

The importance of sequence context in SNP genotyping assay design and performance. *R. Welch^{1,2}, M. Yeager^{1,2}, B.R. Packer^{1,2}, A. Crenshaw^{1,2}, M. Kiley^{1,2}, A. Eckert^{1,2}, L. Burdett^{1,2}, A.W. Bergen¹, S.J. Chanock^{1,3}.* 1) Core Genotyping Facility, DCEG/NCI/NIH, Gaithersburg MD; 2) Intramural Research Support Program, SAIC-Frederick, NCI-FCRDC, Frederick MD; 3) Section on Genomic Variation, Pediatric Oncology Branch, NCI/NIH, Gaithersburg MD.

Millions of SNPs have been deposited in public databases, however, annotations of SNP records in public databases do not always include representation of adjacent flanking SNPs, even if they are known. Such omissions could have a significant impact on the design and performance of SNP genotyping assays, particularly since most current genotyping platforms analyze small fragments of sequence (~100bp). In addition, because genetic diversity is observed among ethnic groups, it is important that the verification and characterization of SNPs and their surrounding sequence include a diversity of ethnic groups. To address these issues, our candidate gene genotyping pipeline includes bi-directional re-sequencing verification of targeted SNPs and characterization of immediately adjacent SNPs in an ethnically-diverse panel of unrelated individuals (N=102) for use in genotyping assay design. An analysis of 1433 target SNPs of interest showed that while 443 (31%) targeted SNPs did not have adjacent SNPs present within an average of 98bp (range 1 to 378), 990 (69%) others had at least one SNP nearby. Furthermore, a substantial number (N=365, 25.5%) of targeted SNPs had at least one of these potentially interfering SNPs present within the expected area of genotyping assay design (within 50bp), even when excluding those SNPs observed in only 1 of 204 chromosomes (mean distance = 23bp, mean MAF = 10.6%). The use of sequence information from public databases for SNP genotyping assay design poses potential problems in assay optimization as well as assay performance (obtaining true genotypes) because adjacent SNPs may interfere with assay performance, leading to genotyping miscalls (i.e. loss of heterozygotes) or assay failure. Such performance issues may contribute to the abundance of false positive results, namely reported studies that do not replicate, since many studies do not use common assays for each interrogated SNP.

High Resolution Human Leukocyte Antigen (HLA) Typing by Mass Spectrometry. *M. Beaulieu, C. Honisch, Y. Chen, D. van den Boom.* Sequenom Inc., San Diego, CA.

The extended collection of genes on the short arm of human chromosome 6 at 6p21.3 is called the Major Histocompatibility Complex (MHC). The locus was named for its role in tissue rejection following transplantation and many of the genes are involved in the biology of the immune system. Because MHC class I antigens are expressed ubiquitously by human leucocytes, they were called Human Leukocyte Antigens (HLA). Structural differences in the HLA molecules expressed by transplant donors and recipients are the major cause of rejection. Underlying these differences is a high degree of genetic polymorphism. During the past 40 years, serological techniques were employed to match potential donors with recipients. The drawbacks of serology include the lack of precision and the need for viable cells. Recently introduced, molecular typing methods require only small amounts of genomic DNA and can offer greater precision. Most molecular methods are derived from locus-specific PCR. Most alleles can be discriminated based on the sequence of exon 2 (HLA class II genes) and exons 2 and 3 (HLA class I genes). Current signal detection platforms are based on gel/capillary electrophoresis and microarrays. These platforms either lack the resolution necessary to distinguish the known alleles, or offer only low throughput capacity. Here we describe a novel molecular HLA typing method based on base-specific cleavage and MALDI-TOF mass spectrometry using the MassARRAY platform. PCR primer pairs for an 863 bp region encompassing exon 2 through exon 3 of the HLA-A locus, and a 234 bp amplicon from the exon 2 of HLA-DQA1 locus, were designed. Twelve (A) and 71 (DQA1) previously typed reference DNA samples were used for initial system validation. The PCR products were submitted to the MassCLEAVE process consisting of an in vitro transcription and base-specific cleavage. The cleavage fragments were analyzed by MALDI-TOF MS. The mass signal patterns of the spectra obtained were cross-compared with sets of theoretical peak patterns of the currently known alleles. Results demonstrate the possibility of using MALDI-TOF mass spectrometry for high-throughput and high-resolution HLA typing.

Expression profiling of candidate genes for brain development and function in neocortex of normal and trisomy 21 fetuses. *T. Haaf¹, G.E. Weiss¹, C. Neuser¹, T. Tralau², U. Zechner¹, D. Galetzka¹.* 1) Inst Human Genetics, Mainz Univ Sch Medicine, Mainz, Germany; 2) Dept Pediatric Pathology, Mainz Univ Sch Medicine, Mainz, Germany.

Human brain development depends on correct spatiotemporal expression of numerous genes. Disturbances of this highly coordinated process are an important cause of mental retardation and other brain disorders. The same set of genes may also contribute to cognitive variation in humans. Identification of genes involved in development of the human neocortex and characterization of their spatiotemporally regulated expression patterns may provide a better understanding of cognitive processes. Microarrays allow monitoring the expression of numerous candidate genes for cognition during fetal brain development in parallel. To this end we have developed a cDNA chip with approximately 600 genes that are known to play a role for brain development and function in humans, mice and/or *Drosophila*, along with 100 control house-keeping genes. This customized gene chip was used to quantify the mRNA expression levels in fetal brain samples from frontal cortex (prospective area A10) between weeks 15 and 25 of gestation. Essentially all control house-keeping genes on the chip displayed detectable expression levels in fetal neocortex. Of the 600 candidate genes tested, approximately 350 showed medium to strong expression and approximately 50 weak expression during fetal brain development. The expression level of a subset of genes changed significantly over developmental time. Approximately 50 genes showed at least twofold expression differences between normal and trisomy 21 brain samples of the same gestational weeks and/or changes in their temporal expression patterns. Some of these genes were reported previously to be expressed differentially in trisomy 21 brains. However, most identified genes, including genes of the MAPK signaling and the Alzheimer disease pathways, have not been associated with trisomy 21 in the literature. Microarray expression data were confirmed by reverse Northern blot experiments. Immunolocalization of the corresponding proteins to specific cell types in fetal brain sections is underway.

Microarray analysis in human and non-human primate brains suggests spatial clustering of the most divergent genes. *M.C. Oldham*^{1,6}, *S. Tsung*², *M. Cáceres*^{3,5}, *J.W. Thomas*³, *S. Horvath*^{2,4}, *T.M. Preuss*⁵, *D.H. Geschwind*⁶. 1) Neuroscience IDP, UCLA, Los Angeles, CA; 2) Dept of Biostatistics, UCLA; 3) Dept of Human Genetics, Emory Univ School of Medicine, Atlanta, GA; 4) Dept of Human Genetics, UCLA; 5) Div of Neuroscience, Yerkes Primate Research Center, Emory Univ; 6) Dept of Neurology, UCLA.

Several groups have employed Affymetrix microarrays to assess the extent to which gene-expression differences in the brain distinguish human from non-human primates. Here we merge two such datasets consisting of 37 human and non-human primate microarrays to increase statistical power (Enard, 2002; Cáceres, 2003). MegaBlast was used to identify all probes with sequence differences between human and chimp, which were removed from the dataset. Global normalization was achieved via MAS 4.0 and expression values calculated using the average difference model. Local normalization was performed for each probe set to eliminate the strong laboratory effect. We identified 336 known, unique genes that were differentially expressed between human and chimp. For each gene, mean absolute divergence (MAD) values were calculated using average expression values from the four species. 150 genes exhibited pronounced expression changes specific to humans. 82% had higher expression levels in human versus chimp, consistent with the human up-regulation noted by Cáceres *et al.* However, the percentage of genes in this list with at least one duplicated coding exon in the human genome (16%) was not significantly different from the percentage found in a list of 13,351 human RefSeq genes (13%), suggesting that duplications alone are not responsible for the observed up-regulation in human (Bailey, 2002). When these 150 genes were mapped to their genomic locations (UCSC), 40% were found within 3 MB of another divergent gene, and almost 20% were found within 1 MB, including 5 pairs of adjacent genes. These findings raise the possibility that changes in shared regulatory regions or chromatin structure affecting the expression of multiple genes simultaneously may have served as catalysts during recent human evolution. Supported by the James S. McDonnell Foundation.

Genetic and Functional Analysis of a Novel Missense Mutation in the Rho-kinase Gene that Potentially Causes Coronary Vasospasm in the Japanese. *H. Kamiunten, G. Koike, J. Mashiba, H. Shimokawa, A. Takeshita, K. Sunagawa.* Cardiovascular Medicine, Kyushu Univ, Fukuoka, Japan.

Background-Coronary vasospasm is one of the major causes of ischemic heart disease (IHD) and its prevalence is known to be higher in the Japanese than that in the Caucasian. Recently, we have shown that Rho-kinase plays a crucial role in coronary vasospasm in humans. However, the genetic impact of Rho-kinase gene (ROCK2) variation on coronary vasospasm remains unknown. Methods and Results-We have recently identified a novel single nucleotide variation, G930T (K310N), in the catalytic domain of Rho-kinase. Japanese patients with vasospastic IHD (n=242) and Caucasian ones from the ENCORE I trial (n=318) were genotyped for this variation, and also for additional 4 polymorphisms of interest, including ACE I/D, angiotensinogen C704T, angiotensin II type 1 receptor A1166C, and eNOS C-786T in each population. The Japanese patients were classified into 3 groups based on their coronary responses to intracoronary acetylcholine administration; large epicardial coronary artery spasm group (LAS), microvascular spasm group (MVS), and normal group (N). The frequency of the T930 allele is higher in the LAS+MVS group (4/129 for the LAS group and 2/59 for the MVS group) than that in the N group (0/54) and the general population (1/98, $p=0.026$) in the Japanese. Interestingly, no Caucasian patients had T930 allele. In addition, CC genotype of the angiotensinogen gene is significantly associated with microvascular spasm. To elucidate whether this ROCK2 variation alters Rho-kinase function, human coronary artery smooth muscle cells were infected with adenoviruses expressing the wild type (G930) or the mutant (T930) Rho-kinase. Western blot analysis revealed a significant increase in phosphorylation level of ERM in cells infected with the mutant Rho-kinase compared to that in cells infected with the wild type after bradykinin stimulation. Conclusions-The G930T variation of ROCK2 has a significant association with coronary vasospasm in the Japanese. The functional analysis demonstrated that this ROCK2 variation could induce hyperresponse to vasoconstrictive stimuli leading to coronary vasospasm.

Identification of candidate genes for congenital heart defects in the NF1 microdeletion interval by expression analysis. *P. Riva, M. Venturin, A. Bentivegna, S. Moncini, R. Moroni, L. Larizza.* Dept Biol & Genetics, Univ Milan, Milan, Italy.

We have previously shown that congenital heart disease is significantly more frequent in NF1 patients with microdeletion syndrome than in those with classical NF1. The outcome of congenital heart disease in this subset of patients is probably caused by the haploinsufficiency of one or more genes in the deletion interval. Following the *in silico* analysis of the deleted region, we found two genes known to be expressed in adult heart, the Joined to JAZF1 (JJAZ1) and the Centaurin-alpha 2 (CENTA2) gene, and seven other genes with poorly defined patterns of expression and function. With the aim of defining their expression profiles in human fetal tissues (15th-21st weeks of gestation), we performed expression analysis by RT-PCR and Northern blotting. We found that HCA66, JJAZ1 and CENTA2 mRNAs were mainly expressed in fetal heart. As at 15th week of gestation heart morphogenesis has mainly been carried out, and due to the unavailability of human fetal RNA from earlier stages of development than those considered, we performed RT-PCR on mouse 7 dpc and 8.5 dpc total embryos, and 10.5 dpc, 12.5 dpc and 14.5 dpc embryonic heart and brain, to identify those that are expressed before during heart development. We found that the orthologous genes Hca66, Jjaz1 and Centa2 mRNAs are also expressed in early stage of development, before (7 and 8.5 dpc) and during (10.5 dpc, 12.5 dpc and 14.5 dpc) the formation of the four heart chambers. By means of bioinformatic tools, we could predict the presence of the binding sites of Nkx2-5, a transcriptional factor expressed early during heart development, in all three mouse orthologous genes, thus reinforcing the hypothesis that they might be involved in heart development and be plausible candidates for congenital heart disease.

Genome Stability Determinants: Diversity of Genes and Mechanisms. *C. Warren*¹, *K. Yuen*², *T. Kwok*², *O. Chen*¹, *P. Hieter*², *F. Spencer*¹. 1) Inst Genetic Medicine, Johns Hopkins Univ Sch Med, Baltimore, MD; 2) CMMT, University of British Columbia, Vancouver, British Columbia.

Proteins and pathways that maintain genome structure have been identified in many genetic screens, and exhibit a high degree of conservation across species. However, traditional mutagenesis and screening methods seldom reach saturation even in model organisms, and more players remain to be identified. The budding yeast deletion collection supports large-scale phenotyping encompassing null alleles for virtually all known nonessential genes. We have examined these mutants using three distinct marker instability assays. Together, these screens detected elevated rates of increased chromosome nondisjunction, loss, mitotic recombination, nonreciprocal rearrangement, and gene conversion. 310 budding yeast genes (6.5% of nonessentials) that contribute to genome stability were identified. Among the 310, annotations on 40% of genes are consistent with known functions contributing to genome stability, 20% are unannotated, and 40% are annotated for functions not previously directly tied to genome stability, such as macromolecular modification and metabolic pathways. 134 of 310 genes exhibit homology to proteins predicted from human, mouse, fly, worm, and/or pombe genomes with BLASTP values $<1 \times 10^{-10}$. The assays exhibited differentiation among the genes identified, with only 10% of genes shared among all three. Studies to determine the mechanisms which distinguish the assay results are underway. This large-scale view of genome stability determinants in a model organism provides a resource for testable hypotheses addressing conservation of protein functions and physiological pathways across eukaryotes..

Association of the mitochondrial T9957C MELAS mutation with Non-Arteritic Anterior Ischemic Optic Neuropathy. *K. Abu-Amero*¹, *T. Bosley*², *S. Bohlega*³. 1) Department of Genetics, King Faisal Specialist Hospital, Riyadh, Saudi Arabia; 2) Neuro-ophthalmology Division, King Khaled Eye Specialist Hospital, Riyadh, Saudi Arabia; 3) Department of Neurosciences, King Faisal Specialist Hospital, Riyadh, Saudi Arabia.

Background: Recently, multiple mtDNA mutations were reported in a group of patients with non-arteritic anterior ischemic optic neuropathy (NAION), raising the possibility that mtDNA changes may be a risk factor for NAION. One patient had a T to C transition at nucleotide 9957, which was previously reported in association with mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS). **Purpose:** To assess the significance of changes in mitochondrial DNA and mitochondrial respiratory function in a man with clinical NAION OU. **Methods:** The patient was examined clinically and with magnetic resonance imaging and spectroscopy. The entire coding region of the mitochondrial genome was sequenced, and mitochondrial function was assessed by flow cytometry after staining with fluorescent dihydroethidium. **Results:** This 76 year-old man did not have MELAS, but he did have seizures beginning at age 69 and bilateral optic nerve injury beginning at age 70. Serum and brain lactate were normal, but mitochondrial functional testing revealed a severe defect in mitochondrial complex III activity. This information was combined with data from a child with MELAS and the same mtDNA mutation. **Conclusions:** The nt-9957 mitochondrial DNA mutation may precipitate both optic nerve and brain injury in some circumstances. Documentation of a mitochondrial metabolic defect strengthens the hypothesis that mitochondrial DNA changes may be a risk factor for NAION.

Correlation of over-expressed IFN-inducible genes with sicca manifestations and autoantibodies in Sjogrens syndrome. *E.S. Emamian¹, J. Leon¹, S.Q. Rao², C.M. Meyer¹, A.N. Leiran¹, E.C. Gillespie¹, T.W. Behrens¹, B. Segal¹, N.L. Rhodus¹, K.L. Moser¹.* 1) University of Minnesota, Minneapolis, MN; 2) Lerner Research Institute, Cleveland, OH.

Sjogrens syndrome (SS) is an autoimmune disorder characterized by lymphocytic infiltration into lacrimal and salivary glands leading to dry eyes and mouth. The etiology of the disease is not well defined. We have used microarray technology to compare global gene expression profiles in peripheral blood samples of SS patients and controls. Initial studies using peripheral blood mononuclear cells indicated that differential gene expression patterns were detectable in SS patients (n=21) compared with healthy controls (n=23). Using three criteria for gene list filtering, 425 differentially expressed genes were identified. A prominent pattern of overexpressed genes known to be induced by interferons (IFNs) was identified. We independently confirmed this gene expression pattern in a second dataset consisting of 16 patients and 22 controls using whole blood samples. Comparison of differentially expressed genes in both datasets revealed a list of 38 overlapping genes, most of which are known to be inducible by IFNs. To assess potential correlations of gene expression levels and clinical manifestations, we evaluated an expanded dataset consisting of 34 patients and 22 matched controls. Clinical variables included erythrocyte sedimentation rates (ESRs), whole unstimulated salivary flow (WUSF), tear flow by Shirmers test (ST), and titers of anti-Ro/SSA and anti-La/SSB by ELISA. Within the SS patient group, each of the five clinical variables were correlated ($p < 0.05$) to genes present in the over expressed IFN-inducible cluster. Anti-Ro titers, anti-La titers, and ESRs were positively correlated to the expression values of the clustered interferon stimulated genes. WUSF and ST values were negatively correlated to these genes, indicating that patients with lower amounts of saliva and tears had higher expression of IFN stimulated genes. These results demonstrate a convincing role for interferon-related pathways in SS and show that expression of these genes is correlated with clinical manifestations.

Cockayne Syndrome: mutation identification using whole genome primer sets. *D. Ginzinger¹, R. Skurat², S. Nidtha³, J.E. Cleaver^{2,4}.* 1) Genome Analysis Core facility, Comprehensive Cancer Ctr, Univ California, San Francisco, San Francisco, CA; 2) Auerback Melanoma Laboratory, Comprehensive Cancer Ctr, Univ California, San Francisco, San Francisco, CA; 3) Applied Biosystems, Foster City, CA; 4) Dermatology & Pharmaceutical Chemistry and Comprehensive Cancer Ctr, Univ California, San Francisco, San Francisco, CA.

Cockayne syndrome (CS) is a progressive neurodegenerative disorder associated with a DNA repair defect. Two genes CSA and CSB, are specifically involved in the CS disorder, and three other genes, XPB, XPD and XPG give rise to various symptoms of xeroderma pigmentosum (XP) or CS according to the particular mutations carried by the patients. The genes involved in CS symptomology are all linked to excision repair of ultraviolet damage (UV) in transcriptionally active genes (transcription coupled repair, TCR). The diagnosis of multigenic diseases like XP and CS are a particular challenge because of the need to repeatedly sequence several genes for detection of potentially novel mutations. The rarity of these diseases, 1-5 per million of the US population means that the potential load for patient diagnosis would be no more than 5 to 10 new cases per month from the whole of the US. Thus, it is difficult to establish a financially sound diagnostic service, especially given the historical need to optimize PCR and sequencing procedures for the coding regions. Re-sequencing is also needed to validate cell lines received from cell banks, because of the possibility of earlier mistakes in assignments. We have employed the new Applied Biosystems VariantSEQr™ resequencing system that uses pre-validated gene-specific primer sets, protocols and analysis software, SeqScape, which incorporates a quality value for each base-call for the detection of individual mutations. Using this system we have quickly and successfully analyzed mutations in 6 cases of CS from a cell bank, validating or correcting previously reported mutations and identifying new ones. This is a preliminary validation study for a 2-gene disease, prior to the greater challenge of re-sequencing XP that has 8 genes. Work supported in part by the Luke OBrien Foundation (to J.E.C).

Study of *RAII* variations in Smith-Magenis syndrome patients without 17p11.2 deletions. *S. Girirajan*¹, *R.E. Slager*³, *C.N. Barth*⁴, *T.L. Newton*⁴, *S.H. Elsea*^{1, 2, 4}. 1) Dept of Human Genetics, Virginia Commonwealth University, Richmond, VA; 2) Dept of Pediatrics, Virginia Commonwealth University, Richmond, VA; 3) Genetics Graduate Program, Michigan State University, East Lansing, MI; 4) Dept of Zoology, Michigan State University, East Lansing, MI.

Smith-Magenis syndrome (SMS) is usually associated with a deletion involving chromosome 17p11.2; however, a previous study from our laboratory showed that SMS in patients with no FISH-detectable 17p11.2 deletion may be caused by dominant frameshift mutations in *RAII* (Nat Gen 33(4):466-8, 2003). These data indicate that haploinsufficiency of *RAII* is likely responsible for the majority of the SMS phenotype, including craniofacial and neurological anomalies. SMS is characterized by distinctive facial features, delayed milestones, cognitive impairment, and mild to severe mental retardation. Behavioral abnormalities include significant sleep disturbances and maladaptive and self-injurious behaviors. We studied 67 putative SMS patients referred to us for evaluation who were first assessed for deletion by preliminary FISH studies using 17p11.2-specific probes. Deletions involving 17p11.2 were not found in 38 of these patients, who were then considered for the *RAII* study. Patient samples were analyzed for variations in the *RAII* coding region and all possible splice variants by a PCR-based sequencing strategy. Interestingly, we identified small deletions, missense mutations, and known SNPs in several of these patients. The number of polyglutamine repeats in all the samples was variable between 9 and 13; no expansions were seen. Some reported SNPs were identified in all patients evaluated. This study has also yielded us with new unclassified synonymous nucleotide changes that are appropriate for the SNP database. With more sequencing based studies, we plan to build a database of all the variants in the *RAII* gene and their relationship to the features of Smith-Magenis syndrome.

Refinement of an 8q Duplication by FISH and CGH in a Patient with Langer-Giedion Syndrome. *M.L. Adam¹, O. Abdul-Rahman¹, J. Milunsky²*. 1) Division of Medical Genetics, Department of Pediatrics, Stanford University, Stanford, CA 94305; 2) Center for Human Genetics, Department of Pediatrics, Department of Genetics and Genomics, Boston University School of Medicine, Boston, MA.

Langer-Giedion syndrome is a disorder characterized by distinctive craniofacial and skeletal abnormalities. Multiple cartilaginous exostoses are seen in virtually all patients by the age of four. Variable mental retardation with significantly delayed onset of speech is seen in most patients. 75% of patients with Langer-Giedion syndrome are found to have a cytogenetically detectable deletion of 8q24.1. This deletion results in loss of functional copies of TRPS1 and EXT1. We present a patient with features of Langer-Giedion syndrome who was found to have a duplication of 8q22.1q23.3, which encompasses the TRPS1 and EXT1 loci. The subject is a 13 year-old Hispanic female born to a 27 year-old primigravida mother at term gestation. Family history was unremarkable. At 4 years, she was noted to have global developmental delay, microcephaly, and dysmorphic features, including a high forehead, bulbous nasal tip, long philtrum, thin lips, and prominent ears. A skeletal survey demonstrated coned epiphyses, madelung deformities of the forearms, and multiple bony exostoses. An initial karyotype was performed and demonstrated a direct duplication of 8q described as 46,XX dir dup (8) (q22.3-q24.13). By age 13, exostoses had developed in the distal radii, scapulae, distal femurs, and right distal tibia with no significant pain. She has severe mental retardation with only 3 words, but has maintained some receptive language skills and remains ambulatory. In an attempt to further characterize her duplication, fluorescence in situ hybridization for TRPS1 and EXT1 was performed and confirmed duplication of both loci. Comparative genomic hybridization was performed and further refined the duplicated region (q21.3q23). It is still unclear how a duplication in this region can lead to clinical features of Langer-Giedion syndrome.

Discordant amplification of genes comprising low copy repeats on 22q11.2 in hominoid species. *M. Babcock*¹, *M. Brenton*², *S. Yatsenko*³, *P. Stankiewicz*³, *J.R. Lupski*³, *J.M. Sikela*², *B.E. Morrow*¹. 1) Molecular Genetics, Albert Einstein College of Med, Bronx, NY; 2) Human Medical Genetics, University Colorado Health Sci Ctr, Denver, CO; 3) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Low copy repeats (LCRs; segmental duplications) constitute roughly 5% of the human genome and are known to mediate chromosome rearrangements associated with mental retardation and congenital anomaly disorders. The 22q11.2 region is particularly susceptible to chromosome rearrangements via LCR22s, leading to deletions, duplications and translocations. LCR22s are composed of blocks or modules containing genes (*USP18*, *BCR*, *GGT*, *GGTLA*) as well as partially duplicated and unprocessed pseudogene copies creating a complex mosaic. Each full-length gene lies in a different LCR22, suggesting varied evolutionary processes. By examining the breakpoint regions in humans, we found evidence for Alu-mediated recombination mechanisms. To trace the evolutionary steps responsible for shaping the LCR22 gene structure in hominoid species, microarray comparative genome hybridization (aCGH) with cDNA arrays, was performed on genomic DNA from human, bonobo, chimpanzee, gorilla and orangutan. If all the hominoid species have a similar number of copies of LCR22 genes, they would be considered stable during evolution. However, we found differences in LCR22 gene copy number, indicating that LCR22s have been quite unstable. Intensity changes were not in proportion to the estimated divergence times of each hominoid species, suggesting that significant amplifications occurred during speciation. Gorilla genomic DNA showed the greatest expansion of LCR22 copy number. Some of the LCR22 genes had similar patterns, indicating that their dosage was altered as a cluster. This was consistent with fluorescence in situ hybridization mapping using BAC clones spanning LCR22s. Quantitative PCR studies using conserved, exon specific probes, are underway. This data suggests that LCR22s have been prone to rearrangements during hominoid speciation. It is possible that similar mechanisms might occur in humans as well, resulting in genomic disorders.

Organization of the regions flanking the centromere of human chromosome 21. *M.R. Bozovsky¹, S.A. Shukair¹, M.R. Cummings², J.L. Doering¹*. 1) Department of Biology, Loyola University Chicago, Chicago, IL; 2) Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL.

It is clear that heterochromatic regions play an important role in genome architecture and maintenance. These regions, which dominate human centromeres, have been largely excluded from the completed genome sequence. Although the sequence requirements for a functional centromere have not been defined, alphoid repetitive DNA clearly plays a role. The D21Z1 alphoid array is a 1.0Mb long, homogeneous array in the centromere of HC21. Using sequence information from the human genome as well as YAC clones which map to 21p, we have begun to determine the sequence organization in the regions flanking the D21Z1 array. The p-arm edge contains a 0.3Mb long satellite-I array that is highly polymorphic in the population. D21Z1 and satellite-I sequences are both found on a 76kb fragment, indicating that these two repetitive arrays are quite close together. Using BAC clone 21B49A22, the last clone in the q-arm sequence which spans both non-repetitive and satellite DNA, our preliminary mapping work has placed the q-arm sequence termination to within 180kb of the D21Z1 array. This region contains a small array of monomeric alphoid DNA that is highly heterogeneous, showing as little as 74% sequence similarity between monomers, and is devoid of both CENP-B boxes and higher-order repeat structure. The monomeric array forms a distinct evolutionary clade from the main D21Z1 array, branching at the root of the tree. This suggests these two arrays have established and evolved independently of each other. Not only does this finding support recently proposed models of centromere evolution, but it also indicates that the monomeric alphoid array may contain HC21 specific sequences. We have subcloned a region of the 21q monomeric alphoid array that is highly polymorphic and which BLAST analysis indicates may be chromosome specific. An HC21 specific centromeric marker would be useful in diagnosing aneuploidy and permit more accurate scoring of a nondisjunction event.

Detection of Microdeletions and Duplications in Human Genetic Disorders Using the Spectral Genomics

Constitutional Chip. *X.Y. Lu¹, R. Locker¹, J.W. Kim¹, W.S. Hutto¹, C. Chinault², A. Patel², A. Beaudet², S.W. Cheung², R.C. Johnson¹.* 1) Spectral Genomics Inc., Houston, TX; 2) Molecular and Human Genetics Department, Baylor College of Medicine, One Baylor Plaza, Houston, TX.

Chromosomal aberrations, such as deletions, duplications and unbalanced translocations, are among the main pathogenic mechanisms that underlie many human genetic disorders. However, conventional chromosome banding is not sensitive (<5Mb) enough to identify subtle chromosome aberrations. Therefore, a rapid and accurate identification of such genetic imbalances could improve diagnosis, which might lead to better treatment. Array-based comparative genomic hybridization (array-CGH) has emerged as a powerful, high-resolution tool, capable of detecting genomic imbalances in one experiment by co-hybridizing differentially labeled test and reference DNA to an array of mapped human genomic DNA fragments (~100-200 kb). We, in collaboration with the Department of Molecular and Human Genetics at Baylor College of Medicine, recently developed a Constitutional Chip which consists of 464 BAC/PAC clones mapped to the telomeric and sub-telomeric region for each chromosome and 41 known constitutional syndromes. We used this Constitutional Chip to study 15 well-characterized, commercially available cell lines derived from patients with known constitutional syndromes. All Constitutional Chip results corroborated previously reported results by FISH and/or conventional G-banding. This study clearly demonstrates the power of the Constitutional Chip in detecting microdeletions and/or duplications without the need for multiple hybridizations using locus-specific probes by FISH. This newly developed Constitutional Chip is a sensitive and rapid diagnostic tool to detect many congenital and constitutional genetic diseases associated with developmental delay and dysmorphism.

Optimization of custom high-density oligonucleotide microarrays for array Comparative Genomic Hybridization (arrayCGH). *R. Selzer¹, T. Richmond¹, N. Pofahl¹, M. McCormick¹, C. Brennen², B. Wittner², B. Khulan³, J. Greally³, L. Chin², R. Green¹.* 1) R&D, NimbleGen Systems Inc., Madison, WI; 2) Dermatology, Harvard Medical School, Medical Oncology, Dana-Farber Cancer Institute; 3) Albert Einstein College of Medicine, Bronx, NY, Department of Molecular Genetics and Medicine (Hematology).

Changes in DNA copy number are one of the hallmarks of the genetic instability common to most cancers and many other diseases. DNA copy number changes include loss of heterozygosity, homozygous deletions, and amplification events. Array-based Comparative Genomic Hybridization (arrayCGH) provides a high-resolution, quantitative method for the assessment of genomic copy number changes. Using NimbleGens maskless array technology, custom 50mer arrays of more than 390,000 oligonucleotides were used to tile at high resolution through a 10 Mb region of mouse chromosome 7, resulting in an average probe density of one 50mer probe every 48 bp, in duplicate. From the resulting tiling data, optimal probes were selected that were able to detect and distinguish both a homozygous (2-copy) and heterozygous (1-copy) deletion within this region. In addition to custom tiling designs, we have optimized a gene-centric whole-human genome CGH array. This design, which includes 26 probes/gene for the 30,391 known and Retseq genes (NCBI build 34, hsg16), was hybridized to DNA samples with known DNA copy number changes. From this array 5 probes per gene were selected that were optimally responsive to changes in target concentration. The result is an optimized oligonucleotide CGH array that is able to detect gene copy number changes from total genomic DNA. On the basis of these results, we anticipate that arrayCGH will become a routine method of high resolution and genome-wide screening for a wide range of chromosomal abnormalities.

Genome-wide Mapping of DNaseI Hypersensitive Sites by Massively Parallel Signature SequencingTM (MPSSTM). *G. Crawford¹, I. Holt¹, J. Whittle¹, S. Luo², D. Zhou², T. Wolfsberg¹, T. Vasicek², F.S. Collins¹.* 1) NHGRI, NIH, Bethesda, MD; 2) Lynx Therapeutics, Inc., Hayward, CA.

The human genome is currently estimated to contain 22,000-25,000 protein-coding genes, but little is known about how most of these are regulated. For over 25 years, mapping DNase I hypersensitive (HS) sites has been used to accurately identify active regulatory elements, but the labor-intensive nature of this technique has limited its application to only a small number of human genes. We have developed a novel protocol to identify gene regulatory sequences on a genome-wide scale by generating a library of DNase HS sites. Approximately 100,000 clones from a library generated from quiescent primary human CD4⁺ T cells were sequenced using MPSSTM, an innovative technology capable of generating 1 million sequence tags per run. Of the clones that uniquely mapped to the genome, we identified 1300 regions that had multiple MPSS clones that mapped within a 500 base pair window. Using a real-time PCR strategy, we determined that 90% of these clusters represent valid DNase HS sites. Over 60% of these clusters do not map within 2kb upstream or 1st introns of known genes, indicating this procedure identifies areas outside of obvious regions known to contain regulatory elements. In fact, some of these sites are greater than 250 Kb from a known gene. By statistical analysis of the frequency of repeat hits to individual DNase HS sites, we estimate there are 100,000 HS sites within CD4⁺ T cells. Almost all of the identified sites in CD4⁺ T cells are also hypersensitive in primary B and CD8⁺ T cells. However, 10% of the DNase HS sites are not hypersensitive in primary hepatocytes and HeLa cells, indicating that this protocol can identify gene regulatory elements that control cell type specificity. A number of these T cell specific DNase HS sites are near genes known to be important in T cell function, including CXCR4, GATA3, and protein kinase C, theta. This strategy, which can be applied to any cell line or tissue, will allow us to better understand how chromatin structure dictates cell function and fate.

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Mapping chromatin structure with ChIP chip experiments. *R. Green*¹, *M. Oberley*², *M. Singer*¹, *P. Farnham*². 1) R&D, NimbleGen Systems Inc., Madison, WI; 2) McArdle Laboratory for Cancer Research, University of Wisconsin Medical School, Madison, WI.

Chromatin Immunoprecipitation (ChIP) chip experiments are a powerful method for determining protein DNA binding interactions. ChIP chip experiments can also be used to define chromatin structure. By using antibodies to modified histones and hybridizing the resulting ChIP samples to custom tiling arrays, we are able to map the chromatin structure of the human genome at unprecedented resolution. We present data showing the location of AcK9 of histone 3, 2MeK9 of histone 3, 3MeK9 of histone 3 as well as RNA Pol II binding across 30 Mb of human genomic DNA. The data show a very high correlation between AcK9 of histone 3 with RNA Pol II binding and a very low correlation between 2MeK9 and 3MeK9 of histone 3 with RNA Pol II binding. The binding of RNA Pol II and the AcK9 of histone 3 also correlate very well with known and predicted promoters. We also present data showing ChIP chip with AcK9 of histone 3 and binding of RNA Pol II can be used to identify new transcripts in the human genome.

Biological Network Analyses: A Current View from Budding Yeast. *F. Spencer*^{1,3}, *B. Peyser*¹, *P. Ye*², *X. Pan*³, *J. Boeke*³, *J. Bader*². 1) McKusick-Nathans Inst of Genetic Medicine, Johns Hopkins Univ Sch of Med, Baltimore, MD; 2) Biomedical Engineering, Johns Hopkins University, Baltimore, MD; 3) Molecular Biology & Genetics, Johns Hopkins Univ Sch of Med, Baltimore, MD.

Biological networks are robust. The mechanisms of robustness can be probed by synthetic lethality, in which it is observed that a cell survives individual loss of function for nonessential genes but cannot survive loss of specific gene pairs. A synthetic lethal interaction provides strong evidence that two genes act in parallel redundant pathways compensating each others' function.

Large-scale synthetic lethality screens are currently being generated for *Saccharomyces cerevisiae*. System robustness observed in synthetic lethality may be defined in two ways: by the synthetic lethal gene pairs themselves, or in gene pairs with similar synthetic lethal interaction sets (congruent pairs). We propose the use of a genetic congruence network to efficiently represent the complexity of genetic interactions. This network supports presentation of genetic interactions at different congruence score cutoff values to accommodate investigation at various levels of network significance.

As susceptibility to common human diseases may encompass gene mutations in multiple pathways, defining the principles of network robustness from analysis of synthetic lethality can provide a framework for understanding disease processes in humans.

The DNA sequence and analysis of human chromosome 11. *T.D. Taylor¹, A. Toyoda¹, Y. Kuroki¹, T. Itoh², Y. Totoki¹, H. Noguchi¹, T. Yada³, A. Fujiyama^{1,4}, M. Hattori^{1,5}, Y. Sakaki¹, Human Chromosome 11 Sequencing Consortium.* 1) Human Genome Research Group, RIKEN Genomic Sciences Center, Yokohama, Japan; 2) Mitsubishi Research Institute, Inc., Tokyo, Japan; 3) Human Genome Center, University of Tokyo, Tokyo, Japan; 4) National Institute of Informatics, Tokyo, Japan; 5) Kitasato University, Sagamihara, Japan.

As a member of the International Human Genome Sequencing Project, our group led the effort for completion of the sequence of human chromosome 11. As major sequence contributor and coordinator, our center took responsibility for analyzing and annotating the entire chromosome (134 Mb).

The current tiling path of chromosome 11 consists of 8 contigs (ranging from 503 kb to 49.6 Mb) containing 1121 accessions. We reached the centromere on both sides and the telomere on 11q. Only six internal clone gaps remain, totaling about 270 kb; continued efforts to close the remaining gaps include long-PCR based on human and chimpanzee whole-genome shotgun data.

Chromosome 11 is relatively gene-dense and harbors genes for several well-known disorders such as: type 1 diabetes, ataxia telangiectasia, and Long QT syndrome. In addition, three medically important regions (IGF2/H19, beta-globin cluster, APO cluster) have been targeted for study as part of the ENCODE project.

The chromosome is being manually annotated according to Hawk standards. The sequence is analyzed by comparing it to all sequence data from other available species, as well as the rest of the human genome. Full-length cDNA and EST sequences are aligned to the sequence in order to try and identify all transcript variants, for both known and novel genes. Several *ab initio* and homology-based gene/exon prediction programs were used in addition to other programs that identify CpG islands, promoter sites, etc. Functional motifs and domains are also identified on the sequence. Comparison with other available genome assemblies will be made to identify additional genes and conserved regions that may be involved in gene regulation. The recent assembly and analysis of the sequence will be presented.

The human X chromosome contains a preponderance of large highly homologous inverted repeats which contain testes genes. *P. Warburton¹, J. Giordano¹, F. Cheung¹, Y. Gelfand², G. Benson²*. 1) Mount Sinai, New York, NY; 2) Boston University, Boston, MA.

The human genome contains a complex pattern of highly homologous genomic duplications. Inverted repeats (IRs) consist of two inverted complementary arms of homologous DNA around a central spacer region. IRs in theory form cruciform structures important in chromosome structure and function. Inverted Repeats Finder (IRF) is novel highly efficient software that can find and index all IRs in the human genome in a few hours on a PC. Repetitive DNA was Repeat Masked during IR detection, but was included in IR extension and alignment. IRF detected 22,624 IRs in the human genome with arm lengths 25bp to 500kb and 75% homology, and spacers 100kb. The 166 IRs detected by IRF with arm lengths 8kb were filtered to exclude IRs in unfinished/ unassembled regions of the genome, or clustered with other closely related IRs, resulting in a robust set of 96 large IRs with an average size of 40kb. 25% of these (24/96) were found to be on the X chromosome, although it represents only ~5% of the genome. Furthermore, 83.3% (20/24) of X chromosome IRs were 99% homologous, compared to only 27.6% (16/58) of autosomal IRs, which suggested that the X chromosome IRs represent either more recent duplications or were undergoing arm to arm homogenization. PCR amplification of both left and right arm/spacer boundaries confirmed at least eight of these IRs in human genomic DNA. Six of these IRs were also present in great apes, suggesting that they are considerably older than their homology suggests. 11 of the 24 X chromosome IRs contained gene families predominantly expressed in testis, including eight cancer-testes antigen genes. These results are remarkably reminiscent of recent findings on the human Y chromosome, which contains 10 large and highly homologous IRs which contain Y-specific testes genes and maintain sequence integrity by gene conversion. The prevalence of large highly homologous IRs that contain testes genes on both the X and Y chromosomes suggest possible roles in controlling gene expression of sex linked genes in the male germ line or during meiotic sex chromosome inactivation.

Standards for Telomerase (TRAP), hTERT, and TaqMan Telomerase Activity Assays. *J.P Jakupciak¹, P.E. Barker¹, W. Wang², S. Srivastava², D.H. Atha¹.* 1) National Institute of Standards and Technology, Gaithersburg, MD; 2) National Cancer Institute, Early Detection Research Network, Rockville, MD.

Cells lose telomeric repeats as they divide in the absence of telomerase. Eventually, telomeres become dysfunctional and cause chromosomal instability that drives the multiple genetic changes required for the formation of a carcinoma. Telomerase activity is a critical factor that increases the replicative potential of tumors. Telomerase activity is the most common biomarker of cancer, characterized in ~90% of all types of cancer. Despite the demonstrated utility of telomerase activity measurements in tumors and in non-invasively collected samples, there is not a clinical diagnostic. Until recently telomerase activity measurement protocols have been low-throughput and not robust enough to accommodate the required statistical analysis to validate telomerase activity as a practical cancer (prediction/early detection) biomarker. Moreover, there is currently no agreed upon standard method for measuring or quantifying telomerase activity. Standards will help to address the extent of telomerase activity specific to the stages of cancer progression and normal tissue. They are necessary as internal/external quality controls to monitor sample integrity or concentration of inhibitors. They will facilitate reliable telomerase activity quantification and help answer if this information is clinically relevant. The EDRN and NIST are developing telomerase standards that function across cancer diagnostic technologies to allow for a normalized and accurate assessment of tumor burden and decisions of response to therapy. To address standardization, we have isolated telomerase and total RNA from the cancer cell line, A549 under different cell cycle states and with different isolation methods. Telomerase was obtained using CHAPS buffer or sonication. We characterized three detection technologies: RAPidTRAP, hTERT RT-PCR, and TaqMan. For example using RAPidTRAP, we compared the variation between single and multiple capillary systems (ABI 310 and 3100). Although these have comparable sensitivity and reproducibility, we found the 3100 to be better suited for sensitivity and high-throughput.

Identification of potential homologues of the human repeat D4Z4, deletions of which are causally associated with facioscapulohumeral muscular dystrophy. *J. Clapp*¹, *J. Fantes*², *J.E. Hewitt*¹. 1) Institute of Genetics, Queens Medical Centre, University of Nottingham, Nottingham, NG7 2UH, UK; 2) MRC Human Genetics Unit, Western General Hospital, Edinburgh, EH4 2XU, UK.

Facioscapulohumeral muscular dystrophy (FSHD) is a dominant human neuromuscular disorder that affects 1 in 20,000 individuals. Typically, FSHD presents in the second decade of life with wasting of facial, upper arm and shoulder muscles. The phenotype can also include retinal vasculopathy, subclinical hearing loss, epilepsy and mental retardation. FSHD is caused by the deletion of an integral number of D4Z4 repeats from a tandem array at 4q35. In the general population there are between 12 and 100 copies of D4Z4 at 4q35; an individual with FSHD has 11 copies. The mechanism whereby this mutation causes FSHD is still debated, but current models favour epigenetic effects such as de-repression of 4q35 genes and altered methylation. D4Z4 contains an open reading frame (ORF) potentially encoding a homeodomain protein. However, DNA hybridization techniques have so far only identified homologues in higher primates, consistent with a non-coding function of D4Z4. Here we show that bioinformatics analysis of draft genome sequences has enabled us to identify potential homologues of D4Z4 in mouse, rat and macaque. The sequence and organisation of mouse D4Z4 (mD4Z4) has been confirmed by a combination of PCR and physical mapping. The mD4Z4 unit is 4.9kb, compared to 3.3kb in human. mD4Z4 contains an ORF of 2025bp, potentially encoding two homeodomains with 55% aa similarity to those encoded by human D4Z4. There is no significant sequence conservation outside the coding region. The mD4Z4 repeat units are arranged in large tandem arrays, with FISH analysis suggesting a single chromosome locus. The rat homologue of D4Z4 potentially encodes a homeodomain protein with 66% aa similarity to the mouse protein. Macaque D4Z4 is longer than human due to an insertion, however the potential ORF is intact. The conservation of the ORF between these species and the identification of several mouse ESTs suggest a coding function for D4Z4. Furthermore, our findings provide potential for the development of a mouse model of FSHD.

Integrated Genetic and Physical Mapping Resources for the Vervet Monkey (*Chlorocebus aethiops*). *N.B.*

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Studies of non-human primates are invaluable for furthering the understanding of a wide range of human traits, especially complex behaviors. The utility of such studies derives from the fact that humans are much more similar to other primates than they are to commonly-used mammalian model organisms such as the mouse. The vervet, or African green monkey, is widely used in biomedical research. We are developing resources to make the vervet monkey a useful model for genetic and genomic research. All resources developed are made available to the scientific community. Tools developed so far include: (1) a large-insert genomic vervet BAC library CHORI-252 (<http://bacpac.chori.org/monkey252.htm>) ; (2) the construction of a vervet genetic map consisting of 378 microsatellite markers; and (3) a first generation, integrated map of microsatellite markers and SNPs of vervet chromosomes 13 and 17, the orthologues of human chromosome 6; Further refinement of the genetic map and expansion to a genome-wide SNP map comprising >5,000 are in progress. We are also developing a web site (<http://www.vervetgenome.org>) summarizing the advances of the project and providing access to the raw data produced by the collaborating groups. Future plans for new resources include large-scale BAC-end sequencing and the creation of physical maps, and the development of brain-specific expression arrays. In addition to development of genetic and genomic tools of immediate use to the scientific community, this project also serves as a model for development of resources for species that are unlikely to be sequenced in the near future.

Wide genome comparisons reveal the origins of the human X chromosome. *H. Hameister¹, M. Kohn¹, H. Kehrer-Sawatzki¹, W. Vogel¹, J.A.M. Graves².* 1) Dept. of Human Genetics, University Ulm, Ulm, Germany; 2) Comparative Genomics Research Group, Research School of Biological Sciences, Australian National University, Canberra ACT 2601, Australia.

Comparative gene mapping between mammalian species has proven the X chromosome to be one of the most conserved gene arrangements, although comparisons with distantly related mammal groups pointed to separate origins of the short and long arms. The recently made available draft sequence of the chicken genome in combination with the genome sequences from pufferfish and zebrafish allows surprising new insight into genome conservation, which can be traced back to the roots of the common vertebrate ancestor 400 to 450 MYA. Most of Xp (Xp11.2-pter including PAR1) is found conserved on chicken chromosome 1q13-q31 in exact colinear order. Proximal Xp and nearly the complete Xq arm including PAR2 are found conserved in a second segment of common synteny on chicken chromosome 4p, but is found in scrambled gene order. This is due to the former microchromosome history in birds of that segment. There are two further small, but gene rich insertions in Xp11.2 and Xq28, which in the fish species can be traced to a common chromosome. The reconstruction, made possible by this combined analysis, favors a model of the ancestral chromosome, which in the mammalian lineage gave rise to the X chromosome, that combines already the later Xp and Xq arm on one chromosome and therefore resembles in a surprising manner the mammalian X chromosome. This ancestral chromosome may have undergone fission in a tetrapod ancestor with fusion of Xp11.2-pter to another chromosome (most probably the later chromosome HSA 21) and refused in the eutherians to the current X chromosome. To our surprise we learn that the X chromosome has been strikingly conserved for 450 million years with relocation to the X of only a limited number of new genes. Therefore, old genes got recruited to further and new functions during evolution. We are studying this scenario by a comparative analysis of human X chromosomal mental retardation, MRX, genes in mammals and birds.

Multispecies comparative sequence analysis of the glucocerebrosidase gene locus. *M.E. LaMarca¹, D.S. Lieber¹, M.E. Portnoy^{2,3}, K.S. Hruska¹, E.D. Green^{2,3}, E. Sidransky¹, NISC Comparative Sequencing Program.* 1) Dept Molecular Neurogenetics, NIMH/NHGRI, NIH; 2) Genome Technology Branch, NHGRI, NIH; 3) NIH Intramural Sequencing Center, NHGRI, NIH, Bethesda, MD.

The human glucocerebrosidase gene (GBA) is located in a gene-rich region of chromosome 1q22, in which 15 genes and at least 2 pseudogenes have been mapped within a 200 kb region. Defects in GBA are the cause of Gaucher disease (GD), the most prevalent lysosomal storage disease. While over 200 mutations in GBA have been identified, genotype-phenotype correlations are limited. Identification of regulatory elements in non-coding regions or in nearby genes and pseudogenes, in conjunction with analysis of the human glucocerebrosidase protein sequence and structure, will contribute to better understanding of the genetic bases of the wide phenotypic variation in this disorder. A target region of approximately 200 kb containing the GBA gene was physically mapped in 8 diverse mammalian species (galago, cow, dog, hedgehog, armadillo, bat, rat and wallaby) and 1 non-mammalian vertebrate (the pufferfish *Fugu rupribes*). Orthologous BACs were isolated and sequenced. For three additional species (chimpanzee, mouse, chicken), orthologous sequence was obtained from the most recent whole genome assemblies. The sequences were aligned to the human genome sequence from July 2003 (UCSC Genome Browser) using MultiPipMaker. Examination of the 58.5 kb region comprising the genes *c11orf2*, GBA, *metaxin 1* (MTX1) and *thrombospondin 3* (THBS3) demonstrated strong conservation of the exonic regions of all four genes in the mammals, but significant alignments in chicken and pufferfish were only seen in THBS3. Screening for Multispecies Conserved Sequences (MCSs) in this region among the mammalian species yielded 77 MCSs at the 95% identity level, 57 in coding regions and 20 in non-coding regions, with an average length of 38 bp. Analysis of the MCS segments and comparative analysis of the translated protein sequences from multiple species will define which Gaucher mutations are in highly conserved regions and whether observed intronic and intragenic variations among patients may also contribute to phenotypic expression.

Clone-based products, high-throughput services and data for functional genomics via GenomeCube. *F. Behrens¹, K. Jürchott¹, P. Neubert¹, B. Drescher¹, E. Rees², B. Korn², J. Maurer¹.* 1) RZPD German Resource Center for Genome Research GmbH, Berlin, Germany; 2) RZPD Heidelberg.

RZPD is a non-profit provider of clones, clone related products and high-throughput services for genome research. In numerous collaborations, RZPD has established a publicly available clone and data collection of more than 35 million clones from 32 different species, and systematically categorizes, verifies and annotates its resources. Increasing demands for optimal clones for various research purposes led to RZPD's **GenomeCube**, a comprehensive and intuitive-to-use database interface. Being gene-based, it provides direct access to biological material and data derived thereof: **siRNA resources.** Several thousand highly gene-specific, long human dsDNAs containing T7 promoters are provided for the generation of highly efficient siRNAs. By the end of 2004, all human genes and about 15,000 genes of mouse and rat will be represented. **Full ORF clones.** Provided in Gateway and Creator shuttle vectors, several thousand human and mouse genes are available as *closed format* clones with natural stop codon, and *open format* clones without stop codon, allowing the generation of C-terminal fusion constructs. **Full-length cDNA clones.** With 2/3 of all human and 1/3 of all mouse genes, RZPD offers an extensive full-length cDNA clone collection including Mammalian Gene Collection (MGC) and The German cDNA Consortium clones. **Verified short cDNA clones.** Ideally suited for microarray production, they are available as genome-wide, non-redundant, sequenced clone collections for human, mouse, and Xenopus. **Genomic clones.** A genome-wide BAC/PAC-clone collection of human origin (24,500 clones, sequence verification in progress) as indispensable resource for matrixCGH and large-scale promoter analysis. **Protein arrays.** High Density Protein Arrays on PVDF membranes, each carrying 27,648 proteins in duplicate, are highly valuable for antibody screening or identification of DNA/RNA binding proteins. In 2003, RZPD became the first genomic resource center worldwide to be granted **ISO 9001:2000** certification stating that RZPD is concerned with product quality, accuracy, and dependability to the benefit of the user.

Program Nr: 1581 from the 2004 ASHG Annual Meeting

Information Visualization of Haplotype and LD Data. *B. Fry*¹, *P. Sabeti*², *D. Altshuler*^{2,3,4}, *J. Maeda*⁵. 1) Aesthetics & Computation Group, MIT Media Laboratory, Cambridge, MA; 2) Program in Medical and Population Genetics, Broad Institute, MIT, Cambridge, MA; 3) Department of Genetics, Harvard Medical School, Boston, MA; 4) Departments of Medicine and Molecular Biology, Massachusetts General Hospital, Boston, MA; 5) Physical Language Workshop, MIT Media Laboratory, Cambridge, MA.

Biology has rapidly become a data-rich science, where the amount of data collected can outpace the speed with which it can be analyzed and subsequently understood. The quantity of this data makes it extremely difficult to gain a "big picture" understanding of its meaning. The amount of data necessitates new software-based tools, and its complexity requires extra consideration be taken in its visual representation in order to highlight features in order of their importance, reveal patterns in the data, and simultaneously show features of the data that exist across multiple dimensions. We have developed a series of tools that examine the Haplotype and Linkage Disequilibrium (LD) data that extend upon current methods for analysis by providing a means for understanding continuously changing, large-scale data sets. Users can quickly move between multiple representations of the same data set, revealing several perspectives on the data in question, while maintaining context between them. These software tools enable researchers to gain an intuitive, "visual" understanding of their data and expose the patterns and relationships found within. <http://acg.media.mit.edu/people/fry/haplotypes/>.

Meta-Analysis of Gross Insertions Causing Human Genetic Disease: Novel Mutational Mechanisms and the Role of Replication Slippage. *J. Chen*¹, *N. Chuzhanova*^{2,3}, *P. Stenson*³, *C. Férec*¹, *D. Cooper*³. 1) INSERM (Institut National de la Santé et de la Recherche Médicale) U613, Génétique Moléculaire et Génétique Epidémiologique, Université de Bretagne Occidentale, Etablissement Français du Sang-Bretagne, Centre Hospitalier Universitaire, Brest, France; 2) Biostatistics and Bioinformatics Unit, Cardiff University, Heath Park, Cardiff CF14 4XN, UK; 3) Institute of Medical Genetics, Cardiff University, Heath Park, Cardiff CF14 4XN, UK.

Although gross insertions (>20 bp) comprise fewer than 1% of gene mutations causing human genetic disease, they nevertheless represent an important category of pathological lesion. In an attempt to study these insertions in a systematic way, 173 gross insertions (132 sequence-known) were identified using the Human Gene Mutation Database (<http://www.hgmd.org>). Some 64% of gross insertions were found to represent sequence duplications of different types (tandem, partial tandem or complex) whilst ~10% were attributable to non-polyglutamine repeat expansions. The duplications ranged in size between 21bp and ~10 kb, were often associated with flanking direct repeats of 2-49 bp, and displayed an inverse relationship between the frequency of insertion and the length of the duplison. Evidence is presented to support the contention that both insertional duplications and triplet repeat expansions are caused by a common mechanism, replication slippage, rather than by unequal crossing over. Some 16% of gross insertions, all 276 bp in length, were found to be due to LINE-1 (L1) retrotransposition involving different types of element (L1 trans-driven Alu, L1 direct, and L1 trans-driven SVA). A second example of pathological mitochondrial-nuclear sequence transfer was identified but appears to arise via a novel mechanism. Finally, evidence for another novel mechanism of human genetic disease is also presented. Thus, whilst gross genomic insertions causing human genetic disease may vary considerably in size, their careful meta-analytical study has revealed extensive diversity in terms of the inserted DNA sequence and has provided new insight into the nature of the underlying mutational mechanisms.

Program Nr: 1583 from the 2004 ASHG Annual Meeting

NCBI and Resources for Genomic Analysis. *D. Maglott, NCBI Annotation Team.* National Center for Biotechnology Information (NCBI), National Library Medicine, NIH, Bethesda, MD.

The National Center for Biotechnology Information (NCBI) of the National Library of Medicine (NLM) at the U.S. National Institutes of Health (NIH) integrates information from numerous sources to support research and understanding of current knowledge of multiple genomes. Information can be accessed by text (symbols, words, phrases, database identifiers), sequence (BLAST), and genomic position in multiple coordinate systems and genomic assemblies (Map Viewer). Tools are also provided to make it easier to take advantage of pre-computed relationships among different types of data (i.e. proteins related by sequence or by conserved domains, homologous genes, PCR primers that appear to be gene-related based on e-PCR or linkage to phenotypes of interest, etc.).

There are several recent developments that will be demonstrated. Text-based queries can now be submitted across multiple databases (Entrez's Global or cross-database search), with related records also being indicated from the Entrez Links menu. Advanced query/Limits pages have been added to key databases such as Map Viewer and Gene to facilitate constructing detailed queries. The number of connections between phenotype and sequence have increased as a result of increased computational and curatorial efforts.

Genomic annotation has been improved by working with external groups to identify members of gene families and/or to compare genomic alignments and gene predictions. We also continue to benefit from the many interested users who identify areas where we can improve our reference sequences (RefSeqs).

Examples of discoveries that can be made using the data and tools will be provided, with emphasis on new features and future directions.

Development of molecular and clinical database resources for human genetics research at The Centre for Applied Genomics. *T.A. Paton¹, J. Herbrick¹, J. Danska^{1,2}, P. Ray^{1,3}, J. Rommens^{1,3}, D. Bulman⁴, L. Osborne^{3,5}, L.-C. Tsui¹, A. Paterson¹, G. Duggan¹, E. Kanematsu¹, J. Zhang¹, J. Cheung¹, R. Khaja¹, T. Tang¹, S.W. Scherer^{1,3}.* 1) The Centre for Applied Genomics, Hospital for Sick Children, Toronto, Ontario, Canada; 2) Program in Developmental Biology, The Hospital for Sick Children, Toronto, ON; 3) Department of Molecular and Medical Genetics, University of Toronto, Toronto, ON; 4) Ottawa Hospital Research Institute, Ottawa, ON; 5) Toronto General Research Institute, Toronto General Hospital, Toronto, ON.

The Centre for Applied Genomics (TCAG; www.tcag.ca) is a Canadian infrastructure established to facilitate research and development in genetics and genomic biology. With the objective of providing core resources and technologies to support large-scale and service-related research projects, five infrastructures operate - (1) Genome Resources, Gene Isolation & Cytogenomics Facility, (2) Genetic Analysis Facility, (3) Microarray Facility, (4) DNA Sequencing and Synthesis Core, and (5) the Bioinformatics and Statistical Analysis Facility. To serve the community TCAG has developed, TCAG curates and hosts several relevant databases to facilitate clinical and basic research in human genetics. The Chromosome 7 Annotation Project database is the most comprehensive resource of genomic, structural, functional and clinical information available for this segment of the genome. Another resource is the Human Genome Segmental Duplication Database - containing information about segmental duplications across the human genome that can be involved in chromosome rearrangements leading to human disease. Descriptions of mutations, polymorphisms and chromosomal rearrangements relating to specific diseases can be found in the Cystic Fibrosis Mutation, Lafora Progressive Myoclonus Epilepsy Mutation and Polymorphism, and Autism Chromosome Rearrangement databases. Most recently, TCAG has developed a database to capture genomic polymorphism data generated from array comparative genomic hybridization (CGH) experimentation. Background on the database structure, curation and other activities will be presented.

Low-copy-repeats database and its application in molecular medicine. *H. Sun*^{1, 2, 6}, *Y.F. Chen*², *P.L. Kuo*³, *S.J. Tsai*^{2, 5, 6}, *C.S.J. Fann*⁴, *K.F. Chen*⁵, *C.C. Lin*⁵, *H.H. Chan*¹. 1) Inst Molecular Medicine, National Cheng Kung Univ, Tainan, Taiwan; 2) Institute of Basic Medical Sciences, National Cheng Kung University Medical College, Tainan, Taiwan; 3) Department of Obstetrics and Gynecology, National Cheng Kung University Hospital, Tainan, Taiwan; 4) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 5) Department of Physiology, National Cheng Kung University Medical College, Tainan, Taiwan; 6) Bioinformatics Center, Center for Biotechnology and Biosciences, National Cheng Kung University, Tainan, Taiwan.

The completion of human genome project has revealed that about 5% of the human genome is comprised of region-specific low-copy-repeats (LCRs). Genomic duplications that lead to chromosomal variations, phenotypic differences and evolutionary changes were well documented. Currently more and more studies have shown non-allelic homologous recombination between LCRs that flanking unique genomic segments may account for significant amounts of human genetic diseases through deleterious loss or gain of genomic segments, which were referred to as genomic disorders. To dissect the structure and content of these LCRs, and study their impact upon human health and genetic diseases, we developed software to search, identify, and analyze region-specific LCRs using sequence information in the human genome database. To further test the application of this information on molecular medicine, specific LCRs on human chromosome 22q11.2, which were commonly deleted in DiGeorge and velocardiofacial (VCFS) syndromes, have been used to design quantitative PCR assay for rapid validation of chromosome 22p11 deletion. Results from this study demonstrated that the LCR database is a good resource to develop rapid approach for molecular diagnosis. In addition, our data suggested that many novel genomic disorders might remain to be identified.

Association of KCNJ11 gene E23K polymorphism with type 2 diabetes in Saudi population. *O. Alsmadi¹, K. Al-Rubeaan², S. Wakil¹, F. Imtiaz¹, P. Carroll¹, M. Rajab¹, S. Al-Katari², B. Meyer¹.* 1) Aragene, Research and Development, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia; 2) Diabetes Center, King Abdulaziz University Hospital, Riyadh, Saudi Arabia.

Polymorphism in KCNJ11, E23K, alters the ATP-potassium channel function by inducing spontaneous over-activity of pancreatic beta cells, thus increasing the threshold ATP concentration for insulin release. Previous studies have shown that the E23K polymorphism is associated with type 2 diabetes in Caucasian populations. Significant variability has been observed with allelic associations in polygenic diseases such as type 2 diabetes. To date there is little or no data on the impact of the E23K polymorphism upon type 2 diabetes in Arab populations. This is particularly so in the Saudi population in which there is a high incidence of type 2 diabetes. We developed a molecular beacon based real time PCR assay for genotyping of the E23K polymorphism. The assay was validated by direct sequencing of a subset of samples. Genotyping was performed in 1162 Saudi patients diagnosed with type 2 diabetes based upon WHO criteria. Genotypes observed were E/E (732;63%), E/K (431;35.5%), and K/K (18;1.5%). Hardy-Weinberg (HW) analysis was performed using this data. Given the incidence of the E and K alleles, the number of heterozygotes (E/K) in the 1162 samples was expected to be 223, whilst that observed was 413. Chi-square analysis indicated the difference between the observed and expected to be highly significant ($p < 0.00001$), indicating deviation from HW equilibrium. The first assumption of HW equilibrium (random mating) is not satisfied by the population studied. However analysis of the same sample set for other polymorphisms showed complete equilibrium. This data supports association of the KCNJ11 gene E23K polymorphism in susceptibility to or pathogenesis of type 2 diabetes in the Saudi population.

Oligonucleotide ligation assay-based SNP genotyping technology allows accurate, cost and time efficient multiplex genotyping of hundreds of SNPs in thousands of samples. *T.M. Frayling, S. McGinn, I.G. Gut.* Centre National de Genotypage, Evry, France.

Comprehensively assessing the role of genes in a complex trait typically requires the genotyping of 100s of single nucleotide polymorphisms (SNPs) in 1000s of samples. Current genotyping technologies do not accommodate this scale of project without large time and cost considerations. We aimed to assess a new technology, the SNPlex System (Applied Biosystems), that genotypes 48 SNPs in a single multiplex reaction for 5000 samples. Reactions are based on oligonucleotide ligation assays followed by PCR with universal biotinylated primers, and separation of hybridised Zipchute markers on capillary sequencers. Four 384 well plates can be processed in 3 person-days at a cost of \$0.05-0.10 using <1ng DNA per genotype. We selected 130 SNPS from the Celera database that randomly covered three type II diabetes candidate genes. In silico searches for incompatible sequences using the SNPlex assay design web-site excluded 36 SNPs, and produced two panels of SNPs. We used 32 CEPH DNA samples from four families (each including 6 meioses) each present in 10 or 12 wells to assess accuracy and reproducibility of the SNPlex assay. Genotypes were tested for compatibility with family relationships using PEDCHECK. Results from a panel of 46 SNPs showed that 42 produced accurate and reproducible results (91%) and that 12 were monomorphic in 32 Caucasian chromosomes, of which 8 (67%) were unvalidated dbSNPs and one unique to the Celera database. For the remaining 30 SNPs 99.8% of 10,860 genotypes were scored. No errors of reproducibility were detected (100%). Three errors of Mendelian inheritance were detected for one marker but later attributed to weak signal strength. Concurrent genotyping of 10 SNPs using Taqman (Applied Biosystems) on a 7900 platform in 30 samples resulted in one discrepancy. In conclusion, the SNPlex System provides an accurate and efficient method of genotyping 100s of SNPs in several 1000 samples. Our study also reflects the likelihood of a SNP being polymorphic when using SNP databases without prior sequence validation and thus evaluates current SNP databases.

Ultra High Sensitivity Ribo-SPIA Amplification Technology for Expression Profiling of Small RNA Samples Using Affymetrix GeneChip Arrays. *G. Deng¹, S. Wang¹, S. Koritala¹, A. Dafforn¹, W. Xiao^{2,3}, R.W. Davis^{2,3}, N. Kurn¹.* 1) NuGEN Technologies, Inc, San Carlos, CA 94070; 2) Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305-5307; 3) Stanford Genome Technology Center, 855 California Avenue, Palo Alto, CA 94304.

Microarray technologies have become powerful tools for global gene expression profiling in drug discovery and clinical research. Limiting factors in such analyses include the RNA quantity required and complexity of protocols for target preparation. The T7-based methods usually require tens of micrograms of total RNA, or multiple rounds of amplification, which are tedious and time consuming, and thus hinder the application of this technology to large scale analysis of precious biologically significant samples. Furthermore, the adaptation of these technologies to clinical small RNA samples will require rapid target preparation methods. The NuGEN's RNA amplification and labeling system called Ribo-SPIA is incorporated in the NuGEN's commercially available Ovation Systems. Ovation Systems are currently suitable for rapid target preparation from 5-100ng total RNA samples. Further enhancement of the Ovation procedure for smaller samples, in the range of 1ng or lower total RNA input will be presented. This new procedure represents a major breakthrough in target preparation for global expression profiling and analysis, potentially down to the single cell level. The major attributes of this procedure are: 1. Higher sensitivity for small RNA samples: This new method generates enough cDNA target by one round of amplification from as little as 1 to 0.1ng total RNA input for hybridization on Affymetrix GeneChip. It has been successfully applied to expression analysis in a variety of clinical samples with limited RNA input from sorted and non-sorted human white blood cells. 2. Simplicity of protocol: The entire target preparation procedure is accomplished in a single day, and is amenable to automation. 3. High sensitivity and reproducibility of array results: GeneChip array results showed excellent reproducibility and high concordance with the standard T7-based target preparation for gene call, and differential gene call.

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The International HapMap Project: Community Engagement. *B. Knoppers.* Dept CRDP, Univ de Montreal, Montreal, PQ, Canada.

The goal of the International HapMap Project is to develop a resource that will describe the common patterns of human genetic variation. 270 DNA samples from four populations are being used, at least initially, to develop the resource: the Yoruba in Ibadan, Nigeria; Japanese in Tokyo; Han Chinese in Beijing; and CEPH (existing samples from Utah residents with Northern and Western European ancestry). No names or medical information were collected, so there is little risk to the privacy of individual donors. However, the HapMap will show which population each sample came from, making it possible to compare patterns of variation among both individuals and populations. This feature raises ethical concerns, including concerns about the potential for group stigmatization or discrimination; concerns about the ability of the HapMap to facilitate population history research (which could lead to findings in conflict with religious convictions or established legal or political claims); and concerns that sampling based on ancestral geography could result in largely socially constructed categories, such as race, being incorrectly viewed as highly precise biological constructs. The Project undertook a community engagement or public consultation process in each community where people were approached for samples. The purpose was to give these people an opportunity to share their views on the ethical, social, and cultural issues the HapMap raises for themselves and their communities, provide input into the way their samples would be collected and described, and offer other relevant input. A Community Advisory Group (CAG) has been organized for each community, so the communities can stay abreast of how the HapMap and samples are being used. While the effectiveness of the community engagement processes and the Community Advisory Groups can only be assessed over time, the experience so far suggests that asking people respectfully about participating in Projects of this type, providing complete, balanced, and accurate information, giving them a chance to express their views, and (where possible) incorporating their input, need not unduly impede research. Instead, it can create a climate in which research proceeds in an atmosphere of openness and trust.

Comparative analysis of the Marsupial *ATRX* and *ATRY* genes. *K. Huynh¹, D.J. Park¹, M. Familiar¹, J.A.M. Graves^{1,2}, M.B. Renfree¹, A.J. Pask¹.* 1) Zoology, The University of Melbourne, Victoria, Australia; 2) Research School of Biological Science, Australian National University, ACT, Australia.

Mutations in the human *ATRX* gene are responsible for the X-linked Alpha-Thalassemia and mental Retardation syndrome. Male patients display varying degrees of gonadal dysgenesis, suggesting that in addition to its role in globin regulation, *ATRX* has a fundamental role in the testis development pathway. In the tammar wallaby (*Macropus eugenii*), there are two *ATRX* orthologues, one on the X chromosome (*ATRX*) and one on the Y (*ATRY*). The full sequence of *ATRX* and *ATRY* is being determined in the tammar wallaby by a combination of RT-PCR, 3' and 5'RACE walking and BAC library screening.

Our investigation into the conservation of a male specific Y-linked *ATRX* homologue in mammals and other vertebrate species by Noahs Ark blot analysis suggest that *ATRY* may have been lost only recently, with the presence of a Y-linked *ATRX* orthologue found in the monodelphis and the seal. This finding further supports the hypothesis that *ATRY* was an important gene in the mammalian sex differentiation pathway that has been supplanted by expression of *ATRX* in the testes leading to redundancy of *ATRY* function [1].

Wallaby orthologue expression analysis has shown that *ATRY* is expressed only in the testis, while *ATRX* retains a broad expression as in humans. Anti-mouse *ATRX* antibodies that cross react with both *ATRX* and *ATRY* in the tammar show *ATRX*/Y protein present in the testis and ovary from day 23 of fetal development (shortly after the appearance of the gonad and well before gonadal sex determination) to the adult [1]. During testis differentiation, *ATRY*/*ATRX* immunostaining was localised in the Sertoli cells, the Leydig cells and the germ cells suggesting that *ATRX*/*ATRY* may play a role in testicular organization.

Reference

1. A.J. Pask, M.B. Renfree, and J.A.M. Graves, (2000) Proc Natl Acad Sci USA 97:13198-13202.

Mapping of a New Adult-Onset Primary Open Angle Glaucoma (POAG) Locus (GLC1G) to Chromosome 5q and Mutation Screening of Candidate Genes. *S. Monemi*¹, *G. Spaeth*², *A. Child*³, *A. DaSilva*¹, *S. Popinchalk*¹, *J. Coppin*¹, *J. Argon-Martin*¹, *R.P. Crick*⁴, *M. Sarfarazi*¹. 1) Molecular Ophthalmic Genetics Laboratory, University of Connecticut Health Center, Farmington, CT; 2) Wills Eye Hospital, Philadelphia, PA; 3) St. Georges Hospital Medical School, London, UK; 4) International Glaucoma Association, London, UK.

Glaucoma is the second cause of blindness with approximately 70 million affected subjects worldwide. In 2003, we presented preliminary evidence for an Adult-Onset POAG locus (GLC1G) on chromosome 5q (ARVO Abst. #1128; ASHG Abst. #2139). The aim of this study was to reduce the GLC1G region and to search for the defective gene in a group of our POAG linked families by candidate gene screening. Our original linkage to the GLC1G locus was identified in 3 POAG families with a total of 29 scoreable meioses, including 16 affected and 2-glaucoma suspects. Recently, a group of 638 individuals (including >400 affected subjects) in 139 POAG families were screened for the entire genome. As this screening also covered the GLC1G locus on 5q, a number of new families showed potential linkage to this region. Additional saturation mapping in these families enabled us to narrow down the GLC1G region to an interval of <5 cM. Since our initial mapping of the GLC1G locus, we have selected 12 potential candidate genes based on their ocular/neuronal expression or their potential protein domains/biological functions and screened them for mutation by direct sequencing of PCR products and/or by SSCP analysis. Several sequence alterations were identified in these 12 genes. However, only one of these variations significantly alters the composition of its encoded amino acid. This alteration perfectly segregates in one of our originally linked families and it is absent in over 470 normal control chromosomes screened by restriction enzyme digestion. Sequencing of this gene in other smaller but potentially linked families is currently in progress. In conclusion, a new Adult-Onset POAG locus maps to 5q and one of the observed sequence variations is segregating in at least one of the GLC1G-linked families. Supported by EY-09947 and M01RR-06192.

Discovering biological relationships in genome-wide expression studies. *W.T. Barry*¹, *A.B. Nobel*², *F.A. Wright*¹. 1) Dept of Biostatistics; 2) Dept of Statistics, Univ of North Carolina, Chapel Hill, NC.

In high-throughput genomic and proteomic experiments, investigators monitor co-expression across a set of conditions in order to gain understanding of the biological phenomena involved. Standard analysis methods test for differential expression of individual genes, yielding a ranked list of significant genes. However, investigators are frequently more interested in finding associations to cellular processes. In order to test hypotheses about the sets of genes associated with cellular processes, investigators have been limited to *post hoc* analyses of gene lists that are potentially biased and subjective by nature. We provide a general framework for conducting valid tests of such gene categories *ab initio* through a 2-stage, structured, permutation-based method, that can be applied to various experimental designs. To illustrate the utility and flexibility of our method, we applied it to a study of human lung carcinomas by Bhattacharjee *et al.* (2001) using gene categories generated from Gene Ontology (GO). Our analysis focused on a subset of the data consisting of microarrays from 16 normal samples and 125 adenocarcinomas. Differential expression of a gene was measured via appropriate "local" statistics for: (1) normal versus adenocarcinoma (t-test), and (2) survival among the adenocarcinomas (Cox proportional hazards model). The Wilcoxon rank sum of local statistics was used as a "global" statistic of expression across an entire category. Empirical p-values and false-discovery rate (FDR) estimates were computed from 10000 permutations. The gene category GO:0016460 'Myosin II' was significantly underexpressed in adenocarcinomas ($p = 0.0012$, FDR = 0.055), while GO:0000786 'Nucleosome' was overexpressed relative to normal samples ($p = 0.0016$, FDR = 0.055). Survival was associated with genes in GO:0005643 'Nuclear Pore' ($p = 0.0001$, FDR = 0.005) with 20 of 30 probesets increasing and 10 decreasing in expression with increased survival. These results illustrate a direct approach for testing differential expression across a gene category and provide novel evidence regarding the involvement of cellular processes in lung cancer development and patient outcome.

Unequal expression assay as a rapid screen for candidate tumor suppressor genes in schwannomatosis. *M.T. Webster, K.E. Larson, M. MacCollin.* Neurology, Massachusetts General Hospital, Charlestown, MA.

Neurofibromatosis 2 (NF2) and schwannomatosis (SCH) are related genetic disorders characterized by the occurrence of multiple schwannomas. Mutations of the *NF2* tumor suppressor gene (TSG) are the molecular events causing disease in NF2 patients, and have also been identified as somatic events in tumors from SCH patients. Linkage and LOH studies have excluded the *NF2* gene as the cause of SCH, and narrowed the *SCH* candidate region which includes a group of genes centromeric to *NF2* on 22q. Since most mutations in TSGs are truncating in nature, we developed a rapid gene-screening assay sensitive to the disproportionate degradation of mRNA harboring truncating mutations. To analyze patients diagnosed with SCH we amplified single nucleotide polymorphisms (SNPs) located in transcribed regions of candidate genes to compare expression of alleles in genomic DNA and cDNA derived from EBV transfected lymphoblast lines. Allelic expression of the *NF2* gene was used as a control. Two *SCH* candidate genes were tested, *CRKL* and *GNAZ* in nine non-founder SCH patients and 4 NF2 patients as controls. Three SCH patients and one NF2 patient were informative for a SNP in the 3' untranslated region of the *CRKL* gene, and all showed equal expression of alleles. Four SCH patients and one NF2 patient were informative for a SNP in exon one of *GNAZ*. Interestingly all showed unequal expression of the cDNA alleles compared to DNA, including the NF2 patient. Subsequent exon scanning of *GNAZ* failed to show molecular alterations outside of the SNP used for screening which was present in SCH, NF2, and normal controls. As expected, NF2 patients showed unequal expression of alleles in the *NF2* gene while expression in SCH patients remained equal. This work illustrates the utility of unequal expression assays in rapidly screening candidate TSGs and excludes both *CRKL* and *GNAZ* as SCH TSGs. We will continue to apply this method in scanning for candidate TSGs located in the *SCH* region.

Retrofitting of an X centromere-based minichromosome with a BAC using Cre/loxP system. *T. Kaname*^{1, 2}, *K. Yanagi*¹, *K. Naritomi*¹, *K. Mann*², *C. Farr*³, *C. Huxley*². 1) Dept Medical Genetics, Univ Ryukyus, Nishihara, Japan; 2) MRC Clinical Sciences Centre, Imperial College London, Hammersmith campus, London, UK; 3) Dept Genetics, Univ Cambridge, Cambridge, UK.

Mammalian artificial chromosomes (MACs) are a potentially useful tool for gene therapy applications as they should allow long-term maintenance of the therapeutic DNA without integration. Minichromosomes derived by telomere-directed truncation of a human chromosome are particularly attractive as a tool for gene transfer because of their mitotic stability. We have established an efficient system for introducing a whole BAC clone including an intact gene unit onto a minichromosome based on the human X chromosome. The use of mutant *loxP* sites with Cre recombinase and direct selection for the retrofitting event led to a high efficiency of correctly retrofitted clones. The 155-kb BAC containing the intact human *HPRT* gene was correctly retrofitted onto the minichromosome in DT40 cells. The *HPRT* gene is expressed from the minigene at approximately the same level in both of these clones. This system allows one to introduce any gene or gene cluster which can be cloned as a BAC into the minichromosome for gene delivery.

Alterations of gene expression pattern in testicular biopsy from a patient with *DAX-1* mutation. *A.L.Y. Pang*¹, *M.M. Martin*², *D.H. Bellan*¹, *A.L.A. Martin*², *O.M. Rennert*¹, *W.Y. Chan*^{1,2}. 1) Laboratory of Clinical Genomics, NICHD/NIH, Bethesda, MD; 2) Department of Pediatrics, Georgetown University, Washington, DC.

Dax-1 is required for spermatogenesis in the mouse. In man *DAX-1* gene products are necessary for normal testicular development and maturation. A patient with the *DAX-1* gene carrying a 11-bp deletion manifested X-linked adrenal hypoplasia congenita (AHC) and hypogonadotropic hypogonadism (HHG). In order to understand the genetic mechanisms of these abnormalities, the pattern of gene expression of the testicular tissue was analyzed using microarrays. Total RNA was extracted from culture cells derived from a testicular biopsy of the patient at 13 years of age. Expressed genes in the culture cells were profiled using Affymetrix HG-Focus GeneChips. The gene expression profile obtained was compared with those from two normal human testicular fibroblast cell lines obtained from ATCC. Among the ~8000 genes examined, 95 displayed 3- or more folds of change in expression levels between patient and normal cells. These differentially expressed genes could be separated into several biological activity groups. Most notably, the comparison expression analyses indicated growth factor genes such as *INTERLEUKIN 1* and *8*, *CHEMOKINE (C-X-C MOTIF) LIGAND 1*, *LEUKEMIA INHIBITORY FACTOR*, and *DIPHThERIA TOXIN RECEPTOR* were significantly downregulated in the testicular cells of the patient. On the other hand, upregulation of expression of genes responsible for prostaglandin biosynthesis (*ALDO-KETO REDUCTASE FAMILY 1, MEMBER C3* and *PROSTAGLANDIN I2 SYNTHASE*) and cell adhesion (e.g. *C-TYPE LECTIN*, *SUPERFAMILY MEMBER 2* and *COLLAGEN, TYPE XV, ALPHA 1*) was observed in the *DAX-1* mutant cells. Other members of the *ALDO-KETO REDUCTASE FAMILY 1*, as well as *FRIZZLED HOMOLOG 7* and *SECRETED FRIZZLED-RELATED PROTEIN 1* and *4* were also increased. These results provide leads for further investigation into the molecular changes that give rise to the clinical symptoms and signs manifested by patients with mutated *DAX-1*.

Evolutionary conservation of regulatory and coding sequences of the pituitary-specific transcription factor,

PROP1. *R.D. Ward*¹, *M.C. Cho*¹, *R.H. Lyons*², *E.M. Rubin*³, *S.J. Rhodes*⁴, *L.T. Raetzman*¹, *T.P.L. Smith*⁵, *S.A. Camper*¹. 1) Dept. Human Genetics, University of Michigan, Ann Arbor, MI; 2) Dept. Biol. Chem., University of Michigan, Ann Arbor, MI; 3) DOE JGI, Walnut Creek, CA; 4) Dept. Biology, IUPUI, Indianapolis, IN; 5) USDA/ARS, Meat Animal Research Center, Clay Center, NE.

All vertebrates have pituitary glands composed of specialized hormone-producing cells. The individual hormones are evolutionarily conserved, although their function varies across the classes of Animalia. Even prochordates such as *Ciona* have pituitary-like hormones, suggesting that genetic regulation of pituitary organogenesis may be conserved among the phylum Chordata. The first known pituitary-specific gene in hierarchy encodes PROP1, a paired-like homeodomain transcription factor. *PROP1* mutations are the most common known cause of multiple pituitary hormone deficiency in humans. Mice homozygous for the spontaneous Ames dwarf mutation or the targeted null allele are excellent models for the human disease. We compared the sequence of the PROP1 gene in five primates, several orders of mammals including species of Rodentia, Carnivora, and Artiodactyla, and related vertebrates such as fish and chicken. A phylogenetic analysis using parsimony supported the orthologous nature of the sequences. Alignment of large regions of genomic sequence from four species and VISTA analysis revealed the presence of several highly conserved noncoding sequences (CNS) near the PROP1 gene. Studies in transgenic mice confirm that one of these is functionally important; it confers a dorsalized expression pattern in the developing pituitary gland. Phylogenetic footprinting across several species implicates specific DNA binding sites in regulation of PROP1 expression. In conclusion, evolutionary comparison has proven to be a valuable approach to understanding regulation of *Prop1*, the key gene for pituitary organ development in vertebrates.

Large scale identification of polymorphisms in Korean population. *B. Oh¹, J-K. Lee¹, H-J. Ryu¹, J-J. Kim¹, J.Y. Lee¹, H. Kim¹, H-Y. Jung¹, G-M. Ryu¹, I. Koh¹, C. Park¹, B. Han¹, J-S. Seo², H.D. Shin³, K. Kimm¹.* 1) Geonmic Epidemiology/Bioin, Natl Genome Research Inst, Seoul; 2) Macrogen Inc., Seoul; 3) SNPGenetics Inc., Seoul.

Genetic variation plays an important role in the multigenic diseases as well as monogenic diseases. Among diverse types of genetic variation the single nucleotide polymorphism (SNP) comprises more than 90%. More than 10 million SNPs have been estimated to be present in human and up to 9 million SNP were so far available in public SNP databases. The analysis of the SNP data has suggested that there are significant differences among populations in terms of SNP pattern. Moreover several issues about how much they are real, what spectrum of the SNP allele frequency distribute on each population and how much the SNPs are representative have been raised. To address these questions, 134 genes in 5q31-33 region have been sequenced to identify SNPs using 24 unrelated Korean individuals and 1,692 SNPs are identified. Among these SNPs 292 SNPs identified from 29 genes have been analyzed. 38% of the SNPs are not found in public database yet, while 67.9% among the novel SNPs have allele frequencies less than 0.1. These results suggest that still more effort should be made to discover SNP to serve as tools in genetic studies of diseases. Ethnic comparison of 27 SNPs reveals that 67% of the SNPs show more than 0.1 difference in terms of allele frequency between Korean and Caucasian, inferring that haplotype map will be significantly different between these. More analysis for the SNPs will be discussed.

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Characterization of Human Variation Resources at the NIGMS Human Genetic Cell Repository. *L. Toji, D. Coppock, P. Bender, C. Beiswanger, J. Beck.* Coriell Cell Repositories, Coriell Inst Med Res, Camden, NJ.

The resources for study of human variation in the NIGMS Human Genetic Cell Repository include a set of panels from diverse populations around the world, the Polymorphism Discovery Resource (PDR), and extended panels of African-Americans, Caucasians, Chinese Americans, and Mexican Americans and recently, in cooperation with NHGRI, panels of cell lines from the International HapMap Project which includes the CEPH population from Utah, USA, the Yoruba people of Ibadan, Nigeria, the Han people of Beijing, China, and Japanese from Tokyo. Lymphoblastoid cell lines and DNA have been prepared for each of the samples in these panels. As part of the routine quality control procedure to verify individual samples in the Repository, the identity of each DNA sample in these panels has been confirmed using a set of six highly polymorphic microsatellite markers. The cumulative profiles for each marker for the existing panels have been compared; the similarities and differences between all of the panels will be discussed along with data on the frequency of the COII/tRNA(Lys) intergenic 9-bp mitochondrial DNA deletion in these populations. In addition, extensive numbers of SNPs have been reported for many of these samples. A review of the availability and extent of SNP data for these panels will be presented. Information about these resources may be found at <http://ccr.coriell.org/nigms>.

Optimal Multiplexing Schemes for 48-plex SNP Genotyping. *Z. Jiang, A. Reyes, R. Oda, J. Mariana, M. Jackson, M. Dobbs, S. Boyer.* Biomedical Research Division, Beckman Coulter, Fullerton, CA.

One of the fruits of the Human Genome Project is the discovery of millions of DNA sequence variants in the human genome. The majority of these variants are single nucleotide polymorphisms (SNPs). Accurate and fast genotyping of SNPs is of significant scientific importance in areas such as linkage/association studies, pharmacogenetics, forensics, and other areas of genetic research. Primer extension, the gold standard technology for SNP genotyping, provides a robust solution at a highly- multiplexed level. The development of multiplex assays in our laboratories involved designing primer sets for both PCR* and primer extension, and examining various combinations of those primer sets and different reaction components under different thermal cycling conditions. In this work, we studied algorithmic approaches to maximizing the multiplexing rate of 48-plex SNP assays and created a primer design engine to provide an efficient tool for assay design. A set of 48 unique sequences was identified and utilized to associate the 48-plex SNP genotyping assays with an oligonucleotide hybridization microarray platform. A standard chemistry protocol based on assays for over one thousand SNPs in the 48-plex format was established. A total of 1152 SNPs were studied on 96 CEPH/Utah family DNA samples from 8 members of 12 families. At 48-plex levels, these SNPs were divided into 24 marker panels: 4 C/A polymorphism panels (192 SNPs), 4 T/A panels (192 SNPs), 4 C/G panels (192 SNPs), 4 T/G panels (192 SNPs), 4 G/A panels (192 SNPs), and 4 T/C panels (192 SNPs). Our results demonstrated over 70 % first pass assay design success, greater than 95% genotyping success rate, and data accuracy of over 99%. This protocol could be applied directly to screen millions of SNPs at a high success rate without further assay optimization. We believe this newly developed technology provides a cheaper and faster solution for automated SNP genotyping with scalable throughput capacity, high assay robustness, and excellent data accuracy. * The PCR process is covered by patents owned by Roche Molecular Systems, Inc. and F. Hoffman La Roche, Ltd.

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Synergistic effects of glycerol in multi-temperature gel electrophoresis dramatically increase the efficiency of SSCP. *K. Li, W. Song, J. Chen, J. Feng, S.S. Sommer.* Dept Molecular Genetics, City of Hope, Duarte, CA.

Single strand confirmation polymorphism (SSCP) is widely used in mutation scanning. To increase detection efficiency of SSCP, a variety of electrophoresis conditions have been evaluated, including glycerol and multi-temperature gel electrophoresis. Mutations in the Factor IX were utilized to optimize conditions. In an initial blinded analysis, nineteen of nineteen mutations were detected with two gel conditions. A pair of generic conditions was then applied to the analysis of the Factor VIII from 48 patients with hemophilia A. In this second blinded analysis, 47 of 47 sequence changes were detected. By loading segments from multiple exons within a lane, it is routinely possible to analyze about a 1,000 segments in a conventional slab gel, which scans a half megabase of sequence (20 segments x 50 lanes per gel, can be analyzed from duplex genomic DNA). We are applying the method to the ABI-377 florescent sequencer, which has the potential to increase throughput by an additional factor of five. While newer technologies capture the imagination, the combination of throughput and high detection efficiency (2.5 megabases analyzed per 2 gels analyzed by the ABI-377) offers a high throughput, low cost approach for scanning for unknown mutations. Throughput may be increased further by capillary electrophoresis.

The characterization of the clinical significance of genetic variants detected in *trans* with known deleterious mutations based upon obvious biallelic phenotypes. *T. Scholl, C. Schwensen, B.C. Hendrickson, K. Eliason, A.E. Deffenbaugh, B. Leclair, C.A. Frye, B.E. Ward, T. Judkins.* Myriad Genetic Laboratories, Inc., Salt Lake City, UT.

A novel method to improve information about genetic variants detected during clinical mutation testing is presented. The approach relies on biological conditions where biallelic mutations induce obvious phenotypes or embryonic lethality. Under these conditions, genetic variants detected in *trans* with known deleterious mutations must be polymorphisms or hypomorphic mutations that convey reduced risk of disease. *BRCA1* was selected to model this approach because testing over 70,000 North American patients has identified over 1,400 genetic variants in this gene. Evidence from extensive clinical testing and mouse transgenic models supports the conclusion that biallelic *BRCA1* mutations induce embryonic lethality. Furthermore, the alleles of *BRCA1* are described by a few prevalent haplotypes, which supports the genetic phasing of variants intrinsic to this method.

Haplotype pairs were assigned to 25,095 anonymous specimens from hereditary breast/ovarian cancer families using SNPs detected during full sequence clinical testing of *BRCA1*. A total of 504 from 1048 genetic variants detected in these specimens could be assigned to one or more haplotypes. There were 21 occurrences of different variants of uncertain clinical significance in *trans* with known deleterious mutations in this dataset. It is likely that these variants convey reduced cancer risk or are benign polymorphisms. From a total of 655 patients that carried these variants in the entire specimen set, there were 352 patients for whom one of these 21 genetic variants was responsible for a test report with clinical uncertainty.

This approach requires large datasets where compound heterozygotes for low prevalence variants and deleterious mutations are found. Present trends of increased genetic testing that involve whole-gene screening should support the application of this technique.

Reducing variability and improving workflow in the collecting, transporting and processing of blood samples for genomic DNA purification using the PAXgene Blood DNA System. *G. Tiller¹, D. Groelz², E. Sittart², C. Lenz², B. Walter-KimmerleZurawinski³, J. Zurawinski⁴.* 1) Div Med Gen, DD2205MCN, Vanderbilt Univ Medical Ctr, Nashville, TN; 2) Research and Development, QIAGEN GmbH, Hilden, Germany; 3) Strategic Marketing Department, PreAnalytiX, Hombrechtikon, Switzerland; 4) Regional Marketing Department, QIAGEN Inc, Valencia, CA.

The PAXgene Blood DNA System provides an easy-to-implement, standardized method for collecting human whole blood samples and purifying genomic DNA. Blood samples are collected in PAXgene Blood DNA Tubes containing a unique additive that maintains sample integrity for DNA purification. Collected blood samples can be stored for up to 14 days at room temperature*, or for longer at lower temperatures. PAXgene Blood DNA Tubes are integrated with the PAXgene Blood DNA Kit, which uses a standardized DNA purification protocol. This protocol was developed to minimize the number of procedural steps and streamline the workflow. The buffer-based protocol minimizes carryover of RNA and protein. The use of a single processing tube per sample reduces the possibility of sample mix-up. The purified DNA is of high yield and quality, and is suitable for use in a range of downstream applications.

*DNA yields may be reduced if tubes are stored at 25C for 14 days or longer.

Proofreading genotyping assays mediated by high fidelity *exo* DNA polymerases. *J. Zhang*¹, *K.T. Yao*², *L.L. Chen*¹, *J.R. Pardinas*³, *D.F. Liao*¹, *K. Li*⁴, *S.S. Sommer*⁴. 1) SNP Institute, Nanhua University, Hengyang, China; 2) First Military Medical University, Guangzhou, China; 3) The Miami Project to Cure Paralysis, University of Miami School of Medicine, Miami, FL; 4) Dept Molecular Genetics, City of Hope, Duarte, CA.

It has been recognized for more than three decades that polymerase fidelity varies according to the presence of proofreading activity mediated by its internal 3' to 5' exonuclease. Polymerases with proofreading function possess high fidelity for DNA replication both in vivo and in vitro, but this well-known class of polymerases has been almost completely neglected in mutation detection in the postgenomic era. The obstacle facing *exo*⁺ polymerases for single nucleotide polymorphism (SNP) detection could be bypassed by the primer-3'-termini modification. This hypothesis has been well tested with the employment of three types of modified allele specific primers with: 3' labeling, 3' to 5' exonuclease-resistance, and 3' dehydroxylation. Accordingly, three new SNP assaying methods have been developed to carry out genome-wide genotyping taking advantage of the enzymatic properties of *exo*⁺ polymerases. One of the three SNP assays, the on/off switch mechanism can be used to discriminate bases upstream of the 3' terminus and thus defines a new concept in de novo sequencing technology. These new mutation detection assays are widely adaptable to a variety of platforms, including multiwell plate and microarray technologies. Application of *exo*⁺ polymerases to genetic analysis, and especially SNP assays, may accelerate the pace of personalized medicine.

Mitochondrial energy metabolism in the klotho KO mouse. *Y. Akita¹, K. Hirata¹, N. Povalko^{1,2}, J. Nishioka¹, K. Ishida¹, M. Nishimura¹, C. Mitsumasa¹, Y. Koga¹.* 1) Pediatrics and Child Health, Kurume Univ. School of Med., Kurume, Fukuoka, Japan; 2) Laboratory of Inherited Metabolic Disease, Research Center for Medical Genetics, RAMN, Russia.

The klotho KO mouse (KKO), a model of premature aging, is characterized by a short lifespan, infertility, arteriosclerosis, hypogonadism, skin atrophy, osteoporosis and emphysema. KKO begin to show growth retardation and become inactive gradually at age of 3 to 4 weeks, and usually die prematurely by age of 8 to 9 weeks. There are several studies of KKO in endocrinology of calcium metabolism and regenerative vascular abnormalities, however, no studies focused on the mitochondrial energy metabolism have been reported before. In the present study, we examined the mitochondrial energy metabolism to investigate the relationship between mitochondrial functions and aging seen in the KKO. We performed histopathological and biochemical analysis of KKO at age of 7 to 8 weeks. Muscle pathology showed a mild variation in fiber size and was smaller diameter than those in wild-type mice. COX stain showed decreased level of enzymatic activities called focal COX deficiency. On electron microscopy, mitochondria were decrease in number and showed abnormal shape in femoral and cardiac muscle. Entire respiratory enzyme activities including Complexes I, II, III, and IV were normal in muscle, however those in kidney were profoundly decreased. Oxygen consumption of isolated mitochondria from kidney suggests that KKO was markedly impaired mitochondrial respiration at the age of 7 weeks. Our results suggest that the disruption of klotho gene causes mitochondrial dysfunction and abnormal energy homeostasis in kidney seen in KKO. The present study showed that function of klotho gene is important to modulate the mitochondrial bioenergetics for the maintenance of mitochondrial homeostasis in mouse.

A rapid microarray based whole genome analysis for detection of uniparental disomy. *O. Altug-Teber¹, A. Dufke¹, S. Poths¹, U.A. Mau-Holzmann¹, M. Bastepe², L. Colleaux³, V. Cornier-Daire³, T. Eggermann⁴, G. Gillissen-Kaesbach⁵, M. Bonin¹, O. Riess¹.* 1) Medizinische Genetik, Universitätsklinikum ,Tübingen,Germany; 2) Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, USA; 3) Hôpital Necker-Enfants Malades, Paris, France; 4) Institut für Humangenetik, Universität Aachen, Germany; 5) Institut für Humangenetik, Universitätsklinikum, Essen, Germany.

To date, uniparental disomy (UPD) with phenotypic relevance is described for different chromosomes. It is likely that additional not yet identified UPD phenotypes exist. Due to technical difficulties and limitations of time and resources molecular analyses using microsatellite markers are only performed in cases with specific phenotypic features. To investigate the feasibility of microarray technology in identifying UPD we carried out a whole-genome UPD screen using GeneChip Mapping 10K Array (Affymetrix) with more than 10,000 single nucleotide polymorphisms (SNPs). Six families, each having an offspring with an initial diagnosis of complete or segmental UPD, were genotyped according to manufacturers instructions. Genotype calls for each SNP were generated with GeneChip DNA Analysis Software 2.0. Clustered biallelic markers homozygous for one allele in the offspring and homozygous for the other allele in the parent who is not involved in UPD were identified as informative markers indicating UPD in the offspring. In two cases with complete UPD (paternal UPD15, maternal UPD7) and in three cases with segmental UPDs (paternal UPD2p with maternal UPD2q, paternal UPD11p and paternal UPD20q) we identified 47, 84, 65, 104, 31, and 13 informative markers, respectively. The accompanying reduction to homozygosity across the chromosome or chromosomal region indicated isodisomy due to a somatic error. In the case of complete maternal UPD15, clusters of homozygous and heterozygous SNPs across chromosome 15 indicated a meiotic error. Since our results demonstrated the presence of UPD as expected and revealed clues about the mechanisms of UPD formation, we conclude that array based SNP genotyping is a fast, cost-effective and reliable approach for whole-genome UPD screening.

A genome-wide functional SNP association study of rheumatoid arthritis. V. Carlton¹, S. Schrodi¹, A. Chokkalingam¹, H. Alexander¹, K. Ardlie², M. Chang¹, J. Catanese¹, D. Leong¹, V. Garcia¹, L. McAllister¹, A. Lee³, E. Remmers⁴, L. Criswell⁵, M. Seldin⁶, D. Kastner⁴, C. Amos⁷, J. Sninsky¹, P. Gregersen³, A. Begovich¹. 1) Celera Diagnostics, Alameda, CA; 2) Genomics Collaborative, Inc, Cambridge, MA; 3) North Shore-LIJ Research Institute, Manhasset, NY; 4) NIH, Bethesda, MD; 5) University of California, San Francisco; 6) University of California, Davis; 7) University of Texas, Houston.

In order to identify non-HLA genetic risk factors for rheumatoid arthritis (RA), we performed a genome-wide, gene-centric, case-control association study (475 cases and 475 individually matched controls) using single nucleotide polymorphisms (SNPs). We tested DNA quality by individually genotyping 87 SNPs from candidate genes/regions, then assembled the DNAs into 32 pools (18-70 individuals per pool stratified by clinical phenotype) and genotyped over 21,000 putative functional SNPs [nonsense, missense, splice site, and transcription factor binding site (TFBS)] in approximately 10,000 genes. To confirm association, all SNPs that show allelic association ($p < 0.05$) are then tested in an independent replication set (840 RA cases in 463 families and 926 individually matched controls stratified into 40 pools). To date >900 SNPs have been tested in the replication study and replicated SNPs ($p < 0.05$ in both studies) have been individually genotyped in both sample sets. The correlation of allele frequencies between individual and pooled genotyping was extremely high and multiple SNPs have been replicated, including numerous SNPs within the HLA region, a missense SNP in a hematopoietic-specific protein tyrosine phosphatase *PTPN22* (p -discovery < 0.0007 , p -replication $< 6E-08$; Begovich et al 2004), a SNP that potentially affects a TFBS in a glutamate receptor (p -discovery < 0.03 , p -replication < 0.02) and a missense SNP in a gene of unknown function on 17q21 that appears to be expressed primarily in T cells (p -discovery < 0.003 , p -replication < 0.008). These results support the proposal that genome-wide association studies, focusing on functional SNPs, are a powerful method for deciphering the genetic basis for complex disease.

Program Nr: 1607 from the 2004 ASHG Annual Meeting

Gene expression profiling of microdissected human breast cancer cells from formalin-fixed paraffin-embedded sections using microarrays. *L. Ding, R. Raja, R. Salunga, S. Chu, E. Cheung, M. Ghosh, M. Erlander.* Arcturus Bioscience, 400 Logue Avenue, Mountain View, California 94043.

Surgical tumor biopsies are commonly preserved as Formalin-Fixed Paraffin-Embedded (FFPE) blocks. Enabling high-throughput gene expression analysis of FFPE samples using microarrays will revolutionize the way tumors are diagnosed and treated. However, since the macromolecules in FFPE tissues are cross-linked, efficient extraction and isolation of RNA with quality adequate for microarray analysis from such samples poses a challenge. We have recently developed and optimized a novel process that integrates efficient isolation of total cellular RNA from FFPE sections, linear amplification of isolated RNA, and synthesis of labeled cRNA for microarray analysis. We have also designed custom oligonucleotide arrays that optimize microarray performance of such samples. We have compared matching frozen and formalin-fixed samples to study the fidelity of gene expression ratios derived from formalin fixed samples and demonstrated that microdissected cells from FFPE sections can be used to study native expression level of genes, and that replicate microdissections of pure tumor cell populations can be utilized to generate highly reproducible expression profiles. Further, we have successfully utilized this platform to identify molecular signatures from breast cancer biopsies, suggesting application to clinical analysis.

Program Nr: 1608 from the 2004 ASHG Annual Meeting

Ultra-high Density Microarray-based SNP Genotyping. *K. Jones, J. Huang, K. Cole, J. Zhang, V. Troung, W. Wei, J. Chen, G. Liu, J. Tom, M. Shapero.* Affymetrix, Inc., 3380 Central Expressway, Santa Clara, CA.

The emerging role of single nucleotide polymorphisms in clinical association and pharmacogenetic studies has created a need for high-throughput genotyping technologies. Synthetic DNA microarrays are an ideal format for high throughput genotyping because they are capable of generating data for >50,000 SNP markers in a highly parallel fashion. The principle challenge is to reproducibly present a subset of the genome to an array such that accurate allelic information is obtained. Kennedy et al. (2003, *Nature Biotechnology*, 21, 1233) and Matsuzaki et al. (2004, *Genome Research*, 14, 414) recently described a methodology termed 'whole genome sampling assay', or WGSA, which utilizes restriction enzyme digestion and PCR to convert genomic DNA into a form that is amenable to highly multiplexed amplification using a single generic primer. This method has been used to genotype 117,000 SNPs in the 30 CEPH trios that are part of the HapMap population collection. We compared approximately 22,000 markers, that had been genotyped in both the HapMap project and using WGSA, in the 90 CEPH individuals. Over 99.6% of the approximately 2,000,000 individual genotypes were called with a concordancy of 99.7%, thereby demonstrating the highly accurate nature of the method. Improvements to the process include the use of 5 um features, which allows for tiling of 250,000 SNPs per array, and the use of alternative restriction enzymes which impacts both the complexity of the target and maximizes the number of SNPs within the target. Lastly, we will present a statistical analysis which will allow one to predict SNP distribution prior to WGSA screening.

Fine mapping of candidate regions for human narcolepsy with high density markers. *M. Kawashima¹, T. Ikuta², G. Tamiya², H. Hohjoh¹, T. Juji³, Y. Honda⁴, H. Inoko², K. Tokunaga¹.* 1) Dept Human Genetics, Tokyo Univ, Tokyo, Japan; 2) Department of Molecular Life Science, Tokai University school of medicine, Kanagawa, Japan; 3) Japanese Red Cross Central Blood Center, Tokyo, Japan; 4) Sleep Disorder Clinic of Seiwa Hospital, Tokyo, Japan.

Human narcolepsy is considered to be a sleep disorder that is affected with multiple genetic and environmental factors. A genetic factor strongly associated with the disorder has been found in the human leukocyte antigen (HLA) region: HLA-DRB1*1501-DQB1*0602 haplotype. As a tool for finding susceptibility regions other than HLA, a genome-wide association study using about 25,000 microsatellite markers with pooled DNAs was performed. The subjects investigated in this study were all Japanese living in the Tokyo area. Allele frequencies in patients and controls were estimated from heights of the peaks detected by an automated sequencer (ABI 3700) with GeneScan software. After the 1st and 2nd screening, 327 microsatellite markers showed differences by mean of at least one statistical method of which forty microsatellite markers were in HLA region due to linkage disequilibrium with HLA-DR, DQ genes. The associations were confirmed with individual samples (cases: 95, controls: 95), and 14 markers still showed statistical differences. Three markers showed strong associations in the analysis using all the available samples (cases: 228, controls: 420, $p < 0.001$). Then, the three candidate regions were subjected to high-density mapping using additional microsatellite markers and were narrowed down to 40-100kb. For one of the candidate region, fine mapping using SNPs with the average distance of 5kb was performed for further narrowing down. As a result, two SNPs close to the microsatellite marker showed stronger associations than the microsatellite marker ($p < 0.0002$). In the region, no known genes but 3 predicted genes were reported in database.

Genotyping over 100,000 SNPs on a Pair of Oligonucleotide Arrays. *R. Mei¹, S. Choudhry², H. Matsuzaki¹, H. Tang³, H. Loi¹, H.-J. Tsai², S. Dong¹, N. Ung², J. Law¹, H. Matallana², N. Battle², W. Rodriguez⁴, S. Narzarrio⁴, J. Casal⁴, G. Marcus¹, S. Walsh¹, M. Shriver⁵, K. Jones¹, E. Ziv², E. Burchard².* 1) Dept Research & Development, Affymetrix, Santa Clara, CA; 2) University of California San Francisco; 3) Fred Hutchinson Cancer Center; 4) University of Puerto Rico; 5) Penn State University.

Single nucleotide polymorphisms (SNPs) are emerging as the marker of choice for a broad spectrum of genetic analyses, and in particular the study of complex human diseases. Here we present a high throughput genotyping platform that uses a one primer assay to genotype over 100,000 SNPs per individual on a pair of oligonucleotide arrays. The selection of SNPs was based on computer predictions of restriction fragments that are likely to contain the SNPs, and further driven by strict empirical measurements of accuracy, reproducibility and average call rate. We estimated call rates > 99%, reproducibility is > 99.99% and accuracy, as measured by inheritance in trios and concordance with the HapMap Project, is > 99.7%. Average inter-marker distance is 23.6 kb, and 92% of the genome is within 100 kb of the SNPs. Average heterozygosity is 0.30, with 105,511 SNPs having minor allele frequencies > 5%. We used the SNP arrays to perform a whole genome admixture mapping experiment for asthma susceptibility loci among Puerto Ricans that are known to be an admixed group with European, African and Native American ancestry. Recently admixed populations such as Puerto Ricans offer potential benefits in association studies since markers informative for ancestry may be in linkage disequilibrium across large distances. We genotyped 30 Native American, 30 West African and 42 European American individuals on the SNP arrays to identify ancestry informative markers which were further used for an admixture mapping study that included 165 Puerto Rican asthmatics with severe asthma (defined as FEV1 < 80% predicted) and 165 matched controls to identifying genes for asthma-related traits in Puerto Ricans. We propose that this novel technology is a promising approach for efficient admixture mapping for complex traits.

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Extracting SNP genotypes from Affymetrix GeneChip DNA Analysis product for analysis. *J. Salit, P. Bonnen, J. Friedman, M. Stoffel, J. Breslow, Affymetrix.* The Rockefeller University, New York, NY.

An integral part of any genotyping effort is to prepare the data for analysis. At The Rockefeller University as part of the on-going genome-wide SNP genotyping of the Kosrae population we have built a software application that extracts data from the Affymetrix GeneChip DNA Analysis product. Using the licensed, object-oriented Java version of the Affymetrix Software Development Kit (SDK) we have created an application with a simple user-interface for converting and filtering the SNP genotype data to text files. This interface allows the user to select the output file formats, the samples to export, and the chromosomal regions that will be used to filter the SNPs. In keeping with the Java environment AffymetrixSNP marker objects are created from the Affymetrix annotation files to hold marker information like chromosomal location and the mapping of SNP calls to nucleotides. RUSubject objects store the personal attributes of each sample like gender and parents as well as a collection of AffymetrixGenotype objects that store each genotype call and know how to format that call for different export options. A CHPReader knows how to get the contents of an Affymetrix CHP file and extract the SNP genotype calls for the sample associated with that CHP file. These and other objects work together with the Affymetrix SDK to bridge the pipeline between genotype data generation and analysis by creating the analysis input files.

Testing the Centurion GeneChip Technology as a Platform for Genotyping 100,000 SNPs. Z. Xue¹, L.P. Briley¹, S.A. Shouse¹, K.M. Long², M.G. Moore², K.L. Nangle², D.P. Yarnall¹, J.D. Briley¹, D.D. Kelly¹, D.Z. Zaykin¹, K.G. Au³, A.C. Gatherum¹, M.G. Ehm¹, S.L. Chissoe¹, A.M. Saunders¹, N.K. Spurr¹, E.H. Lai¹. 1) Discovery & Pipeline Genetics, Glaxo Smith Kline, RTP, NC; 2) Bioinformatics, Glaxo Smith Kline, RTP, NC; 3) Genomic & Proteomic Sciences, Glaxo Smith Kline, RTP, NC.

The Affymetrix GeneChip Mapping 100K Set is a pair of oligonucleotide microarray chips that offers the ability to generate more than 100,000 SNP genotypes simultaneously for one subject from 500 ng of genomic DNA at a cost of ~\$0.01 per genotype. We tested the pre-production technology called Centurion as a method for genotyping 100,000 SNPs at once. In this pilot study, 30 CEPH subjects and four duplicates were genotyped on the chips in 3-4 weeks. An average of more than 111,500 SNP genotypes were called per subject. Genotypes from four duplicate samples typed on different chips were greater than 99.99% concordant. In this small Caucasian sampling, there were approximately 8,100 SNPs that were out of Hardy-Weinberg equilibrium ($p < 0.001$) and about 10% of the SNPs were monomorphic. While additional testing is necessary and currently underway, the GeneChip Mapping 100K Set has the potential to be a fast, accurate, cost-effective means of genotyping 100K SNPs at once.

High Stability Vectors for Cloning Trace Amounts of Unstable DNA. *H. Burrell, R. Godiska, M. Patterson, D. Mead.* Lucigen, Middleton, WI.

Large sections of eukaryotic, viral, and microbial genomes are refractory to cloning with conventional host-vector systems. This apparently unclonable DNA results in cloning gaps, rearranged sequences, or a complete inability to clone particular regions, especially those that are AT-rich or encode toxic peptides. Most plasmid vectors induce transcription and translation of inserted fragments, which is responsible for much of the DNA instability. The pSMART series of transcription-free cloning vectors were developed to alleviate these problems. The utility of these vectors was demonstrated by stably cloning several classes of substrates that were unstable or unclonable in conventional vectors, such as:

- Telomeric repeats and other AT-rich fragments from *Pneumocystis carinii*.
- >10 kb clones of *Tetrahymena thermophila* genomic DNA(75% AT).
- Toxic regions of the mouse hepatitis virus genome.
- cDNAs from a fish cardiac muscle library.
- Strong promoters from phage lambda.

Additional methods were developed to clone trace amounts of DNA from uncultured viruses and environmental phage. Unidentified base modifications have been accommodated by these methods, as the genomes of previously unclonable marine bacteriophage and several unculturable bacteria have been cloned. Nearly undetectable amounts of Inoue-Melnick virus were cloned and sequenced using these methods. Further improvements are being undertaken to clone genomic DNA and cDNA from single cells.

The entire mitochondrial DNA analysis of 9 MELAS patients with an A3243G mutation. *I. Kaori¹, Y. Akita¹, N. Povalko^{1,2}, K. Hirata¹, J. Nishioka¹, M. Nishimura¹, C. Mitsumasu¹, Y. Koga¹.* 1) Pediatrics and Child Health, kurume university school of medicine, Kurume, Fukuoka, Japan; 2) Laboratory of Inherited Metabolic Disease, Research Centre for Medical Genetics, RAMN, Russia.

MELAS (Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes), one of the subtype of mitochondrial disorders, is caused by the mutation of mtDNA (mitochondrial DNA). Investigating all the pathogenic mutations or polymorphisms of mtDNA which affects biochemical functions is important to consider the genotype-phenotype correlation in each patient at molecular level. We have analyzed 9 MELAS patients who were diagnosed clinically, muscle-histochemically, and genetically. The mitochondrial DNA was extracted from the blood or muscle biopsy specimens from patients using previously described methods. We have analyzed the entire sequence of mtDNA by described methods. The complete mitochondrial genome was amplified by long-PCR method in 7 overlapping fragments using 14 primers and followed by the ExoSap-IT treatment (USB Corporation, USA), and CEQ Dye Terminator Cycle Sequencing kit (Beckman Coulter, Inc.). The sequences were assembled in a contig using the program DNASIS Pro (Hitachi Software Engineering Co, Ltd, Japan) and the resulting contig was aligned to the Cambridge sequence. We have also analyzed any base change by designated a primer-set and suitable restriction enzyme using PCR-RFLP methods. All MELAS patients have an A3243G mutation in the mitochondrial tRNA^{Leu}(UUR) gene. We have found 13 mtSNPs in Non-coding regions, 10 in ribosomal regions, 2 in tRNA regions (including A3243G), and 47 in protein-coding regions. Among 9 MELAS patients, one has a heteroplasmic nonsense mutation in the COX II gene in addition to the A3243G. This patients showed denovo mutation according to the genetic analysis in the family members and showed more severe clinical phenotype among our MELAS patients. We have successfully constructed an Automated Sequence of the entire mitochondrial genome, and found 72 mtSNPs in 9 MELAS patients rapidly and effectively.

Re-sequencing as a precise method for estimating SNP allele frequencies in pooled DNA. *V. Kodavali¹, A. Northup¹, S.A. Bacanu², J. Wood¹, P. Levitt³, K. Mirnics⁴, D.A. Lewis⁵, B. Devlin², V.L. Nimgaonkar¹.* 1) WPIC, Univ Pittsburgh, Pittsburgh, PA, USA; 2) Department of Psychiatry, University of Pittsburgh, Pittsburgh, Pennsylvania, USA; 3) John F. Kennedy Center for Research on Human Development and Department of Pharmacology, Vanderbilt University, Nashville, TN 37232, USA; 4) Department of Psychiatry, University of Pittsburgh, School of Medicine, E1453 Biomedical Science, Pittsburgh, Pennsylvania 15261, USA; 5) Department of Psychiatry, University of Pittsburgh, 3811 O'Hara Street, W1651 BST, PA 15213, Pittsburgh, USA.

To evaluate re-sequencing as a tool for estimating allele frequencies from pooled DNA samples, we measured peak heights from re-sequencing traces. Twenty six known SNPs were initially genotyped in pools from 200 controls and 200 patients with schizophrenia (frequencies of less common SNP alleles: 1.75% - 50%). These estimates were compared with frequencies determined from individual genotypes of the same samples using an accurate allele-specific extension method based on the ligation reaction (Illumina Inc.). There was a highly significant correlation between the two methods for individual SNP allele frequencies ($r = 0.984$, $p = 0.001$, $n = 26$ SNPs, 52 pools), as well as case-control differences ($r = 0.958$, $p = 0.001$, $n = 26$ comparisons). The standard deviations (SD) for quadruplicate assays were comparable to other methods for pooled genotype assays (mean 0.018, range 0 - 0.109). SD values for separately prepared pools using 50 samples were comparable to the larger pools, when 10 SNPs were analyzed separately (mean 0.018, range 0.005 - 0.076). The re-sequencing method provides a reliable, economical, single step process for surveying genomic regions, detection of SNPs and estimation of allele frequencies. Pooled DNA samples thus provide a precise method both for detecting SNPs and for estimating their frequencies in large cohorts. By obviating the need for genotype assays, it also provides economy.

Identification of rare β -thalassemia mutations and Hb South Florida by genomic sequencing in the Malay, Chinese and Indian ethnic group in Malaysia. *J.A.M.A. Tan¹, P.S. Chin¹, Y.C. Wong¹, K.L. Tan¹, E. George², L.L. Chan³.*

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Malaysia's population of 22.7 million comprise of Malays 47.2%, Chinese 24.9%, Indians 7% and other races 13.9%. Beta-thalassemia is common in the Malays and Chinese with a heterozygous carrier rate of about 4.5%. Five common β -globin mutations present in the Chinese - FS 41/42 (-CTTT), IVS2-654 (C-T), -28 (A-G), CD 17 (A-T) and FS 71/72 (+A) - account for 92% of β -thalassemia. Four rare β -mutations at CD 26/Hb E (G-A), CD 15 (G-A), Cap (+1) (A-C) and -29 (A-G) account for a further 4% of β -thalassemia in this race. In the Malays, three β -globin mutations at CD 26/Hb E, IVS1-5 (G-C) and IVS1-1 (G-T) account for 73% of β -thalassemia while six other β -mutations at FS 41/42, IVS2-654, FS 8/9 (+G), CD 17, CD 19 (A-G) and Cap (+1) account for a further 12% of the identified mutations. Thus, using the ARMS and Gap-PCR, rapid and specific confirmation of β -globin mutations can be identified in 96% and 85% of the Malaysian Chinese and Malays respectively. Genomic sequencing of a 904 bp region of the β -globin gene (294 bp upstream of the Cap site to 113 bp downstream of the IVS2-1 site) was carried out to identify the uncharacterized β -alleles. Six rare β -globin mutations and one hemoglobin variant were confirmed: in the Malays - FS 16 (-C), IVS1-1 (G-A), IVS2-1 (G-A), -86 (C-G), Hb South Florida (CD 1, G-A); in the Chinese - FS 27/28 (+C), CD 43 (G-T, 2 patients) and in the Indians -88 (C-T). These rare β -mutations had been reported in very low frequencies (3%) in different ethnic groups/populations, except for -88 (observed in the American Blacks at 21.1%). Hb South Florida does not cause any significant clinical problems and has been previously reported only once in a Caucasian family. In heterozygotes, Hb South Florida has been reported to be present in levels of 12-20% and about 20% of the protein is acetylated at the NH₂ terminus of the β -chain.

From large-scale RT-PCR cloning to identification and characterization of alternative splicing. *J.Q. Wu, S. Hulyk, A. Garcia, Y. Yuan, A. Sneed, C. Kowis, E. Fuh, K. Haeberlen, I. Yakub, J. McPherson, P. Gunaratne, R.A. Gibbs.* Human Genome Sequencing Ctr, Baylor Col Medicine, Houston, TX.

Alternative splicing plays a major role in the production of diversity in the human proteome. Alternative splicing patterns have served as prognostic markers and drug targets in human diseases including cancer. Although analysis of expressed sequence tag (EST) and microarray data has provided useful information about alternative splicing, it can not be determined whether the splicing of multiple variable regions with an individual transcript is coordinated. Only sequencing full length cDNA can resolve these cases. In previous publication, we have described a genome-wide RT-PCR cloning pipeline to collect full-length cDNAs. This pipeline also allows us to capture novel splice variants: about 50% of subclones generated from our pipeline have sizes different than the corresponding Refseq transcripts. We searched for the subclones that have end sequences matching the targeted genes. This resulted about 2000 subclones that are potential splice variant. We have full-length sequenced 133 subclones, and observed 42 deletion clone and 18 insertion clone. In order to confirm the alternative splicing events, junction specific primers have been designed for 41 potential splice variants and the corresponding Refseq transcripts of each gene. About 35% novel splice events were verified by RT-PCR and direct sequencing. In order to explore the biological significance of the alternative splicing events, we selected genes implicated in cancer, such as WISP1, to characterize their relative expression level in cancer tissues to adjacent normal tissues by quantitative real-time PCR. Alternatively spliced transcripts show cancer type specific up- or down- regulation. Our results implicate the variation of protein domains potentially influences protein function, and the ratio of alternative transcript / Refseq transcript may underlie the cancer mechanism.

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Resequencing of 2.5 MB of the ENCODE region in 48 individuals from 4 ethnic groups. *I. Yakub, H. Arredondo, S. Doyle, J.Q. Wu, A. Hodgson, L. Lewis, X. Wei, D. Muzny, D. Wheeler, J. Belmont, R. Gibbs.* Human Genome Sequencing Center, Baylor Col Medicine, Houston, TX.

As part of the International HapMap project, the Human Genome Sequencing Center at Baylor College of Medicine has resequenced a 2.5 MB of the ENCODE region in 48 individuals from 4 different ethnic groups consisting of Caucasians, Yorubans (Nigeria), Chinese and Japanese. The 2.5 MB region consists of five 500 KB segments on chromosomes 7p15.2, 8q24.11, 9q34.11, 12q12 and 18q12.1. In order to span this entire 2.5 MB region using 750bp amplicons, we designed 8340 primers to generated 4170 amplicons. Using a multiplex (6-plex) PCR strategy, we carried out 66,720 PCR reactions and setup 400,320 sequencing reactions to resequence all 48 individuals. To meet these needs, we established a re-sequencing pipeline that includes large-scale primer design, automated PCR and sequencing reactions in 96-well and 384-well formats, data management tools and automated data-analysis and SNP detection software. The data obtained from this study is allowing us to determine the smallest haplotype blocks in this region and give us an estimate of the SNP density needed to obtain an accurate haplotype map of the genome. We will also be able to determine the amount of variation in this region among different ethnic groups, frequency of rare variants- both synonymous and non-synonymous and SNP density in relation to gene structure. A subset of the novel SNPs discovered in this study will be further genotyped in 270 individuals from the four different ethnic groups.

Genotyping performance of whole genome amplified DNA derived from genomic DNA templates of variable quantity and quality. *A.W. Bergen^{1,2}, Y. Qi², K. Haque², M. Beerman², C. Glaser², I. Barrow², J. Hartwell², R. Welch², M. Garcia-Closas¹, P. Castle¹, N. Rothman¹, S. Chanock^{1,2}.* 1) Division of Cancer Epidemiology and Genetics, NCI, Bethesda, MD; 2) Core Genotyping Facility, NCI, Bethesda, MD.

The promise of whole genome amplification (WGA) is that genomic DNA (gDNA) need not be a limiting factor for molecular genetic analysis. We evaluated the performance of commercially available MDA WGA protocols using gDNA derived from lymphoblastoid cell lines (N=27), buccal cells from cytobrushes (N=20), and buccal cells from expectorated mouthwash (N=48 matched irradiated and unirradiated samples) to provide both non-degraded and degraded gDNA inputs. An input range of 1-100ng of gDNA into WGA was used. Analysis of wgaDNA was performed using three DNA quantification methods and N=15 STR and N=49 loci SNP genotyping assays performed in duplicate on every gDNA and wgaDNA sample for N=17,632 STR and N=104,486 SNP genotypes attempted. These analyses of wgaDNA were designed to characterize the yield and composition of the wgaDNA, genotyping completion and concordance rates, and the samples and assays that failed to produce genotypes from wgaDNA. Relative amounts of double-stranded and human-specific wgaDNA increased with increasing gDNA input. Genotyping performance of wgaDNA was significantly reduced compared to that of gDNA. For example, non-degraded gDNA STR/SNP completion rates were 100%/99.7%, and STR completion rates were more severely affected (95% or 64%) than SNP completion rates (96% or 81%) by reduced gDNA quantity and quality (1ng non-degraded or degraded gDNA, respectively). Increasing amounts of gDNA template improved wgaDNA genotyping performance, though not optimally for wgaDNA derived from degraded gDNA. Reduced wgaDNA genotyping performance was primarily due to the increased variance of allelic amplification, resulting in LOH or increased undetermined genotypes. Other variables affecting wgaDNA genotyping performance included increased %GC content and WGA reagent source. Optimal genotyping performance of wgaDNA was not obtained without at least 10ng of non-degraded gDNA template.

Comparison of buccal cell collection methods on DNA yield and quality. *J. Chang, K. Heard, C. Sanders, G. Satten, K. Steinberg, M. Gallagher.* CDC, Atlanta, 30341.

Buccal cell collection is a noninvasive, economical, and convenient means for obtaining DNA for clinical and research studies. However, unlike whole-blood specimens, buccal swabs yield varying amounts and quality of DNA. We evaluated DNA yields from buccal swabs collected by two methods. With method 1, study participants used cytology brushes to self-collect buccal swabs. Participants then placed the swabs in the manufacturers plastic storage tube and mailed them to us. With method 2, nurses either collected or supervised the self-collection of buccal swabs by participants using cytology brushes. The nurses sprayed the collected swabs with alcohol. No significant differences existed between the average total DNA yields of two groups, although 10.6% of the self-collected samples had a concentration 0.1 ng/L, while none of the nurse-collected samples were below this concentration. To further evaluate the quality of DNA in these samples, we performed microsatellite analysis of D7S1797 and D13S317 in triplicate on 109 nurse-collected and 113 self-collected buccal swabs. All 109 of the nurse-collected specimens were successfully amplified in triplicate for both microsatellite markers; the replica results were 100% concordant. In contrast, only 101 of the 113 self-collected specimens were positive for both microsatellite markers in all three replicate reactions. Of the 101 repeat-positive specimens, 10 yielded discordant results due to allelic dropout: 6 had discordant D13S317 results, 1 had discordant D7S1797 results, and 3 had discordant results for both microsatellite markers. DNA concentrations 0.1 ng/L were strongly associated with amplification failures and allelic dropout ($p=2 \times 10^{-7}$, D7S1797; $p=7 \times 10^{-7}$, D13S317). One of the major differences between the two collection methods was the alcohol treatment of the swabs in the nurse-collected group. This treatment may serve to reduce bacterial growth that may cause DNA degradation. Although collection of buccal swabs by mail is convenient and economical, poor-quality specimens may have a significant effect on downstream applications. Thus, the method of collection should be carefully considered.

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TaqMan-based Real-time Quantitation of MicroRNAs. *C. Chen, D. Ridzon.* Genomics R&D, Applied Biosystems, Foster City, CA.

We developed a new quantitative real-time assay for mature microRNAs (miRNAs) that employs the specificity of ligation, and the sensitivity and accuracy of standard real-time 5' nuclease assays. A total of 24 miRNA targets were successfully designed and tested. Ct values were highly correlated to the input target quantity ($R^2 = 0.998$) over six orders of magnitude. The level of miRNA expression ranged from 0 to 4,814 copies per cell in seven different tissues or cell lines from human and mouse. Of 24 miRNAs examined, mir-16 is the most abundant, particularly in the thymus. Similar patterns of miRNA expression were observed in human and mouse. These results are consistent with previously published Northern data. In addition, no effect of background genomic DNA on quantitation was found. In summary, this TaqMan-based real-time quantitation of miRNAs is fast (~3 h), specific, accurate, and sensitive, allows for high-throughput screening of all possible miRNAs, and can be performed from as little as 1 ng of total RNA. Possible applications of this new technology include the validation of *in silico* predicted miRNAs and miRNA profiling in tissue or cell samples within or between species.

A Whole Genome Quantitative Association Study Using Data from the HapMap Project. *J.C. Engert¹, L.J. Oksanen², M. Lemire², B. Ge², S. Gurd², D. Shah¹, T.J. Hudson^{1,2}, T. Pastinen².* 1) Depts Medicine & Human Genetics, McGill University, Montreal, PQ, Canada; 2) McGill University and Genome Quebec Innovation Centre, Montreal, PQ, Canada.

We have performed a large-scale survey of quantitative variation that is associated with specific haplotypes that have been determined by the HapMap project. The single nucleotide polymorphism (SNP) alleles from the Utah portion of HapMap project were scored from the lymphoblast cell lines (LCLs) of 30 CEPH trios. Thus, if we examine quantitative traits in the unrelated parents of these trios (the exact cell lines from which was derived the HapMap data), our experiments are the equivalent of performing a whole genome association scan in 60 individuals. Our criteria for quantitative trait selection included stable, well characterized traits, that are preserved in lymphocytes. We believe that these cells can be an appropriate cell model for a variety of human metabolic systems. Approximately 600,000 SNPs are now available from the HapMap project for genomewide studies with approximately 400,000 having an allelic frequency of at least 0.025. Our initial experiment was to examine the mRNA level of the low density lipoprotein receptor (LDLR) as a quantitative trait. We have previously demonstrated heritability of mRNA levels in pedigrees from CEPH families. In addition, one of the key responses to statin treatment is an increase in LDLR and this response has been shown to be preserved in lymphocytes. We will present data from baseline LDLR as well as after statin treatment. Combining the detailed genetic information provided by the HapMap from human cell lines with biomedically relevant quantitative phenotypes collected from these same cell lines will demonstrate great utility. This approach should identify many new candidate genes/regions and this technique should be applicable to many complex traits and their associated biochemical pathways.

High-throughput genetic, epigenetic and gene expression analyses of cancers using an integrated BeadArray technology. *J. Fan¹, M. Bibikova¹, E. Wickham¹, E. Chudin¹, J. Yeakley¹, L. Zhou¹, D. Barker¹, M. Chee¹, Z. Lin², J. Floros², N. Thomas², Y. Wang², C. Seifart³, M. Kostelec⁴, J. Modder⁴, T. Downs⁴, J. Wang-Rodriguez⁴.* 1) Illumina, Inc, San Diego, CA; 2) Penn State University, Hershey, PA; 3) University of Marburg, Germany; 4) VA San Diego Healthcare System, San Diego, CA.

We have developed a versatile BeadArray technology for high-throughput genomic analysis in large populations. The arrays are made of beads located in microwells of optical fiber bundles or silicon slides. Both formats support multi-sample analysis with the optical fiber bundles formatted into a matrix that matches a 96-well microtiter plate and the silicon slides divided into discrete sites compatible for loading multiple samples on to a single silicon slide using a multi-channel pipette. A series of cancer studies are conducted on this system: (1) 1152 cSNPs derived from 377 cancer related genes are genotyped with paired gDNA and RNA isolated from 96 lung cancer tissues and 30 cancer cell lines. As a result, a comprehensive genetic and allele-specific expression profile is created. In addition, a targeted LOH and gene amplification analysis is carried out at these genes, coupled with a genome-wide scan using 4600 highly polymorphic SNPs. (2) DNA methylation status is monitored for 375 CpG sites located in the promoter regions of 143 genes in 104 lung cancer and normal samples. Multiple squamous cell carcinoma specific and adenocarcinoma specific methylation markers are identified ($P < 0.001$) and further validated in an independent sample set ($N=46$). (3) Expression profiles of 512 candidate genes are generated for 240 formalin-fixed, paraffin-embedded prostate carcinomas and 60 benign hyperplastic prostates with at least 5 years of clinical follow up, and are used to identify specific molecular markers that correlate with the following clinical parameters: tumor type and grade, organ confined disease vs. locally advanced tumors, therapeutic response and overall prognosis. These projects should help correlate genetic, epigenetic and gene expression profiles to the most important clinical parameters in a large set of clinical samples.

Identification of disease susceptibility genes using the CEQ 8800 Genetic Analysis System. *E. Hayashi*¹, *H.-C. Chi*², *S.K. Boyer*², *D.W. Still*¹. 1) Department of Horticulture/Plant & Soil Science, California State Polytechnic University Pomona, Pomona, CA; 2) Biomedical Research Division, Beckman Coulter, Inc., Fullerton, CA.

Whole-genome scans have proven to be a powerful and efficient method to identify diseases caused by single genes using microsatellite and single-nucleotide polymorphism (SNP) analyses. However, the majority of diseases are effected by complex *gene x gene* and *gene x environment* interactions. The mapping of such factors is complicated by the population structure of the sample populations and spurious associations can easily be made. In genome-wide association studies it is necessary to test at least thousands of SNPs because of the limited amount of linkage disequilibrium in the human genome. Further, because of the difficulties attributed to mixed populations and the inability to control environments in human population studies, alternative approaches to mapping human diseases have been developed. These include the mapping of quantitative trait loci (QTL) on genetically distinct organisms, such as mice and rats. The aim of QTL analysis is to detect linkage between the phenotypes and genetic markers and to assign levels of significance to each detected QTL. Most often SNPs and microsatellites are used in these studies, but other marker technologies can be used as well. The CEQ 8800 Genetic Analysis System is capable of generating a variety of data that can be used to map QTLs associated with phenotypes. We present data using a model organism and discuss several approaches by which marker scaffolds can be utilized to facilitate identification of QTLs and determine the conservation of the QTLs in non-model organisms in comparative genomic studies.

A quality control platform for spotted 70-mer microarrays. *M. Hessner*¹, *L. Meyer*¹, *V. Singh*², *T. Zahrt*², *X. Wang*¹.
1) Dept Pediatrics/Endocrinology, Medical College of Wisconsin, Milwaukee WI; 2) Dept Microbiology/Molecular Genetics, Medical College of Wisconsin, Milwaukee WI.

Spotted 70-mer arrays offer potentially greater specificity and an alternative to laborious use of cDNA libraries. Since microarray fabrication is a considerable source of data variance, we previously directly tagged cDNA probes with fluorescein allowing for prehybridization visualization and development of array quality control (QC) thresholds. Likewise, labeling of oligonucleotide probe sets could offer the same benefits, however, this practice is currently cost-prohibitive. Therefore, a *Staphylococcus aureus*-derived, fluorescein-labeled tracking oligonucleotide, was added at low-molarity into the printing buffer to allow indirect monitoring and optimization of fabrication variables for rat and *Mycobacterium tuberculosis*-specific oligonucleotide arrays. The tracking oligonucleotide did not effect hybridization efficiency or introduce ratio measurement bias in hybridization data. Linearity between the mean log Cy3/Cy5 ratios of genes differentially expressed from arrays either possessing or lacking the tracking oligonucleotide was observed ($R^2=0.90$, $p<0.05$). There were also no significant differences in Pearson's correlation coefficients of ratio data between replicates possessing (0.72 ± 0.07), replicates lacking (0.74 ± 0.10), or replicates with and without (0.70 ± 0.04) the tracking oligonucleotide. ANOVA analysis confirmed that the tracking oligonucleotide introduced no bias. Titrating target-specific oligonucleotide (40 μ M to 0.78 μ M) in the presence of 0.5 μ M tracking oligonucleotide, revealed a fluorescein fluorescence inversely related to target-specific oligonucleotide molarity, making tracking oligonucleotide signal useful for quality control measurements and differentiating false negatives (synthesis failures and mechanical misses) from true negatives (no gene expression). Therefore, a slide qualification strategy, as well as putative array and spot acceptance criteria have been developed. Finally, this novel, inexpensive, approach is useful for more sophisticated post-hybridization applications including automated image analysis and data filtering.

DNA isolation and amplification from Formalin Fixed, Paraffin Embedded Tissues. *K.E. Jones¹, A.A. Camargo², P. Simpson³, R. Deadman¹.* 1) GE Healthcare, Piscataway, NJ; 2) Laboratory of Molecular Biology and Genomics, Ludwig Institute for Cancer Research, So Paulo, Brazil; 3) The Breakthrough Toby Robins Breast Cancer Research Centre, Institute of Cancer Research, London, England.

Large scale genetic analysis studies require the availability of large amounts of genomic DNA. Patient tissues are frequently preserved in formalin and embedded in paraffin for long term storage and ease of transport. However, genomic DNA extracted from these sources is limited in quantity and poor in quality, making it insufficient for whole genome analysis. Current DNA extraction methods generally yield short DNA fragments and it is only possible to PCR amplify regions of less than 1 kb from the extracted DNA. PCR-based whole genome amplification methodologies such as degenerate oligonucleotide primed PCR (DOP-PCR) have not provided an adequate solution to the problem. Recently, an alternate approach has been introduced. Genomic DNA is amplified by multiple-primed linear amplification using the highly processive, strand displacing enzyme Phi29 DNA polymerase. This poster will describe an evaluation of genomic DNA extraction methods from paraffin embedded formalin fixed tissue and amplification using Phi29 DNA polymerase.

Studying human populations using 100,000 genome-wide SNPs on synthetic DNA microarrays. *G. Kennedy¹, M. Cao¹, M. Kennedy¹, M. Taub²*. 1) Genomics Collaborations, Affymetrix, Santa Clara, CA; 2) Department of Statistics, UC Berkeley, Berkeley, CA.

We used a recently described genotyping technology (Kennedy et al. 2004) to rapidly genotype >116,000 SNPs (including 2,334 SNPs on the X chromosome) in a variety of human and ape DNA samples. We constructed haplotypes using Merlin (Abecasis et al. 2003) and studied recombination patterns in 3-generational CEPH families. We found larger recombination fragment lengths and higher variability on the X chromosome (mean=41 Mb + 57Mb) than on autosomes (mean=24 Mb + 28 Mb) but found no major differences in fragment length or variability between males and females. We calculated Wrights F_{st} statistic, a measure of population structure, for SNPs in three human populations (Caucasian, Asian and African-American). When comparing African and non-African populations we found evidence for significantly increased F_{st} values on the X chromosomes relative to the autosomes. In contrast, we found no statistically significant difference in F_{st} values between X and autosomes for the two non-African populations. We assigned ancestral alleles to 69,500 SNPs by genotyping chimpanzee and gorilla DNA on the human microarrays. LD analysis indicates that many of the high- F_{st} SNPs on the X chromosome are located in blocks consisting of non-ancestral alleles that have achieved high frequency. These data suggest that large regions on the X chromosome may have undergone selection and are consistent with the hypothesis that migration of Caucasian and Asian populations out of Africa may have been accompanied by a population bottleneck or other demographic event(s). Genotyping using high-density microarrays provides a rapid and facile method for determining allele frequencies in various populations and for studying recombination patterns at high resolution in pedigrees.

A novel method for genotyping the angiotensin 1-converting enzyme insertion/deletion polymorphism utilizing the MGB™ Eclipse system and dissociation curve analysis. *D. Koontz, S. Nikolova, M. Gallagher.* CDC, Division of Laboratory Science, NCEH, Molecular Biology Branch, Atlanta, GA.

Angiotensin I-converting enzyme (ACE) is important in blood pressure regulation and electrolyte balance, and its influence on the susceptibility to coronary heart disease is being extensively studied. The insertion/deletion (I/D) polymorphism in intron 16 of this gene accounts for much of the interindividual variability of plasma ACE concentration. To date, the most commonly used methods for genotyping this polymorphism involve conventional PCR, nested PCR, or PCR-RFLP followed by gel-based fragment size analysis. These methods are labor intensive, require a large quantity of DNA, are prone to mistyping, and are not easily automated for studying large numbers of samples. We have developed sensitive insertion-specific and deletion-specific assays using the MGB™ Eclipse probe system (Epoch Biosciences) with subsequent dissociation curve analysis to accurately and reliably detect all three genotypes (DD, ID, and II). This hybridization-triggered fluorogenic probe system utilizes a minor groove binding ligand that forms hyperstabilized duplexes with complementary DNA. The reaction occurs in a single well, uses nanogram amounts of DNA, and requires only endpoint analysis of results. We analyzed DNA from three CEPH families to demonstrate consistency with Mendelian inheritance. In addition, the assays were performed on 50 anonymized DNA samples from volunteers and 90 samples from the Coriell Polymorphism Discovery Resource designed to reflect the diversity in the human population (European American=27%, African American=27%, Mexican American=13%, Native American=6%, Asian American=27%). No significant deviation was found in expected genotype distributions when compared with other reports. All results were found to be 100% concordant with conventional PCR. Here we present a rapid, sensitive, and accurate nongel-based method which, unlike similar assays involving the use of SYBR Green dye, is highly specific with the use of gene-specific probes. The method is easily adapted to any automation platform for the analysis of the ACE I/D polymorphism in large sample populations.

A NOBLE SNP TYPING METHOD BASED ON DNA COMPUTING. *N. Nishida*¹, *K. Hirayasu*¹, *M. Takasu*¹, *A. Suyama*², *K. Tokunaga*¹. 1) Department of Human Genetics, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan; 2) Department of Life Sciences, The University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo, Japan.

As a consequence of Human Genome Project and SNP discovery projects, a number of SNPs, which include susceptibility SNPs for common diseases, have been revealed. There has been a great urge to develop a highthroughput SNP typing method with high accuracy and low cost. Here, we show a noble SNP typing method based on DNA computing. In the DNA computing based analysis, we prepared internal codes which we call DNA coded numbers (DCNs). DCNs have optimum properties in physical and chemical aspects so as to execute each DNA computing process precisely. DCNs consist of three parts; named SD, D1 and ED. SD and ED are commonly prepared at both edges over all DCNs and are used for common priming sites in PCR. D1s are different among DCNs and are used to identify SNPs of interest. During the SNP typing by DNA computing, the information of genotype is converted to one of DCNs by one to one manner using oligonucleotide ligation assay. After the encoding reaction from genomic information to DCNs is accomplished, PCR is executed to increase the signal intensity and then SNP genotype is read out by detecting the type of DCNs using capillary array. There are some advantages in DNA computing based method. To use the same set of well-designed DCNs enables us to perform SNP typing with high accuracy. Oligonucleotide ligation assay, engaged in the encoding reaction, enables us to execute multiplex SNP typing. The same set of DCNs is universally used for all types of application such as gene expression profiling and SNP typing, ensuring low cost analysis of genomic information.

Novel genes associated with risk and age-at-onset in Parkinson disease identified through iterative association mapping. *S.A. Oliveira, Y.J. Li, X.J. Qin, S. Züchner, M.A. Nouredine, M.A. Hauser, T. Skelly, S. Ebersviller, M.A. Pericak-Vance, J.M. Vance.* Duke Center for Human Genetics, Duke University Medical Center, Box 2903, Durham, NC.

We previously reported genetic linkage for age-at-onset (AAO) in Parkinson disease (PD) to a region on chromosome 1p (LOD=3.41). To identify the gene(s) associated with AAO and risk in this region, we first tested genes derived from the genomic convergence approach (genes significantly differentially expressed in the substantia nigras of PD cases versus controls which map to the chromosome 1 linkage region). Association mapping was the next step, but has potentially very high costs. To minimize these costs, we performed what we term iterative association mapping, where we first genotyped single nucleotide polymorphisms at an average distance of 100 kilobases over a 20 megabase region under the AAO linkage peak and then gradually increased the density of markers (50 kb, then 25 kb, etc) as needed to identify areas of significant association. Our data set consists of 284 multiplex families with 574 affected and 641 unaffected individuals. The average AAO \pm SD of affected individuals is 59.9 \pm 12.7 years (range: 14-90 years; 58.3% males). After correction for multiple testing, we identified several polymorphisms significantly associated with AAO in PD.

Using dbSNP to Maximize the Success of Custom Arrays for the Illumina Bead Lab SNP Genotyping Platform.

E.W. Pugh¹, R. Ingersoll¹, P. Boyce^{1,2}, G. Ingersoll³, M. Farkas¹, J. Pettengill¹, C. Bark¹, I.A. McMullen¹, T. Templeton¹, S. Carney¹, J. Gearhart¹, K.F. Doheny¹, Y.Y. Tsai¹, C. Boehm¹, J.L. Goldstein¹, L. Watkins¹, A.F. Scott¹.
1) Institute of Genetic Medicine, Johns Hopkins Univ, Baltimore, MD; 2) Illumina, San Diego, CA; 3) Genetic Software Innovations, Cicero, NY.

The SNP Center provides high throughput SNP genotyping using the Illumina Bead Array technology. Most projects have required the development of custom panels of SNPs. We hope to identify criteria for the selection of SNPs most likely to succeed using information available from dbSNP. We present preliminary performance data from 5229 SNPs. Validation status information was obtained from dbSNP.

Success and Quality of SNP Assays by dbSNP Validation

		----- All SNPs -----			Polymorphic SNPs--	
Validation	n	%polymorphic	%monomorphic	%fail	%called	50%_GC Score
no info	131	48.85	6.11	45.04	99.61	0.73
frequency	146	54.11	2.74	43.15	99.49	0.74
cluster	226	62.83	4.42	32.74	99.51	0.78
doublehit	1316	81.61	1.52	16.87	99.69	0.78
Hapmap	3410	87.27	1.09	11.64	99.88	0.80

The proportion of failing assays decreased with increasing levels of validation present in dbSNP. Markers with better validation were also more likely to be polymorphic. For polymorphic assays, the average call rate and 50th percentile GenCall Score, a measure of genotype quality, increased slightly by the validation type of the SNP, but these differences were not statistically significant.

Genome-wide identification of functional non-coding elements by digital analysis of chromatin structure. *P.J.*

*Sabo*¹, *M. Hawrylycz*¹, *R. Humbert*¹, *J. Wallace*¹, *A. Kohli*², *Q. Li*², *M. Yu*², *M. McArthur*¹, *J. Stamatoyannopoulos*¹. 1) Dept. of Molecular Biology, Regulome, Seattle, WA; 2) Div. of Medical Genetics, Univ. of Washington, Seattle, WA 98195.

Comprehensive identification of sequences that regulate transcription and other chromosomal processes is one of the major goals of genome biology. We developed a novel stochastic methodology - Digital Analysis of Chromatin Structure (DACS) - for comprehensive, genome-wide mapping of DNaseI hypersensitive sites (HSs) and associated cis-regulatory sequences *in vivo*. We used 19-20bp genomic DNA tags to localize individual DNaseI cutting events in nuclear chromatin, and analyzed >250,000 tags from erythroid cells. DACS tags can be accurately and comprehensively (>99%) mapped to the human genome without the aid of a restriction site scaffold, providing base-pair resolution. We applied a quantitative algorithm to discriminate statistically significant tag clustering over small genomic intervals, and show that such clusters identify active chromatin elements corresponding to both known and novel functional elements. A unique feature of DACS is that it permits unbiased evaluation of the chromatin accessibility of sequences from disperse genomic loci at a sensitivity heretofore not obtainable. We observed repeated tagging of specific regulatory sequences far in advance of other well-described elements. This suggests the existence of a discrete hierarchy of chromatin accessibility in the nucleus, compatible with physical compartmentalization. An *in silico* simulation confirmed that DACS could be extended to effect localization of the majority of DNaseI hypersensitive sites (and hence classical cis-regulatory sequences) in the human genome, without requiring a separate molecular validation step for each element. Overlaying a comprehensive cis-regulatory map on emerging genetic variation data has the potential to accelerate greatly discovery of functional non-coding polymorphisms that underlie inter-individual variability in gene expression and quantitative traits.

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Multiplex genotyping of 384 to greater than 100,000 SNP loci per DNA sample on the BeadArray platform. *M.R. Shen, D. Campbell, W. Chang, J. Chen, D. Doucet, F. Steemers, C. King, J. Musmacker, H. Ren, K. Kuhn, M. Chee, J.-B. Fan, K. Gunderson, A. Oliphant.* Illumina, San Diego, CA.

SNP genotyping is increasingly the technology of choice across a broad spectrum of genetic studies, including candidate gene and fine-mapping studies, family-based linkage, and high-resolution, genome wide association scans. To accommodate the variety of study designs, there is a need to efficiently span a wide range in the numbers of SNP loci interrogated and samples processed. Therefore, we developed a highly flexible array-based platform that enables SNP genotyping at a broad range of multiplex levels. We also developed two genotyping assays with complementary capabilities. The GoldenGateTM assay uses a universal array and can accurately process hundreds of samples each day. The assay generates a multiplex pool of up to 1,536 SNP-specific PCR amplicons, each of which contains a different hybridization sequence complementary to one of 1,536 universal capture sequences on the array. Because universal capture sequences are designed into the oligos, no changes to the array are needed for new assays. To date, over 250,000 highly accurate GoldenGate SNP assays have been developed as part of the International HapMap Project (www.hapmap.org). The WGG assay is described in an accompanying abstract (Steemers et al, submitted). The Whole Genome Genotyping (WGG) assay is optimal for genotyping many thousands to hundreds of thousands of SNPs at a time, and can also accommodate a wide range in the number of samples. The WGG assay does not use PCR and can directly analyze genomic DNA of mammalian complexity. Therefore, for practical purposes, there is no limit on multiplexing. This capability is unique among SNP genotyping assays. Together, the GoldenGate and WGG assays allow study designs to use as few as 384 to well over 100,000 SNPs. Both approaches provide robust, highly accurate genotyping with excellent call rates. Details of the implementation of the two assays will be described, including the process flow logic and examples of how key parameters can be varied to accommodate different studies.

Sequence-specific localization of functional non-coding elements in vivo by quantitative chromatin profiling. *J. Stamatoyannopoulos¹, M. Hawrylycz¹, R. Humbert¹, J. Wallace¹, M. Yu², M. McArthur¹, M. Dorschner¹*. 1) Dept. of Molecular Biology, Regulome, Seattle, WA; 2) Div. of Medical Genetics, University of Washington, Seattle, WA 98195.

Identification of functional non-coding elements that regulate transcription and other basic chromosomal processes is a major goal of genome biology. Localization of functionality to specific sequences is a requirement for genetic and computational studies. Here we describe a generic approach - Quantitative chromatin profiling (QCP) - that uses quantitative, high-resolution analysis of in vivo chromatin structure over an entire gene locus to effect rapid and precise localization of cis-regulatory sequences and other functional elements. To demonstrate the sensitivity and specificity of QCP, we analyzed ~330kb of human genome sequence from 7 diverse gene loci and cleanly delineated a spectrum of classical elements including enhancers, promoters, insulators, and locus control regions. QCP provided exceptional sensitivity (100%) and specificity (>99.6%) for validated functional elements within these loci. In addition to defining the core regions of many elements with greater resolution, QCP also demonstrated the ability to identify novel elements in heavily-explored genomic territory such as the beta- and alpha-globin loci, the T-cell receptor-alpha locus, and the X-linked photoreceptor locus. We show further that analysis of genetic variation within elements identified by QCP can be used to determine the role of specific regulatory sequences in modulation of a model quantitative phenotype. Systematic, high-throughput, high-resolution identification of functional non-coding sequences surrounding candidate genes for major common disorders should significantly expand our knowledge of regulation of these genes and simplify the search for genetic variation affecting quantitative traits.

Assessment of a novel flow-through porous microarray for the rapid analysis of gene expression profiles. *Y. Wu¹, P. de Kievit¹, L. Vahlkamp¹, D. Pijnenburg¹, M. Smit¹, P.J. Boender¹, C. Ingham¹, A.K. Raap², A.B. Chan¹, R. van Beuningen¹.* 1) PamGene International BV, s-Hertogenbosch, The Netherlands; 2) Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands.

A novel microarray system that utilizes a porous aluminum-oxide substrate and flow-through incubation was developed for rapid molecular biological testing. To assess its utility in gene expression analysis, we determined hybridization kinetics, variability, sensitivity and dynamic range of the detection using amplified RNA. To show the feasibility with complex biological RNA, we subjected Jurkat cells to heat shock treatment and analyzed the transcriptional regulation of 23 genes. We found that trends (regulation or no change) acquired on our platform are in good agreement with data obtained from real-time quantitative PCR and Affymetrix GeneChips. Additionally, our results demonstrate a linear dynamic range of 3 orders of magnitude and accelerated hybridization kinetics (10-fold compared to conventional microarrays). The minimum amount of transcript that could be detected in 20 l volume is 2-5 attomol, which enables the detection of 1 in 300,000 copies of a transcript in 1 g of amplified RNA. Hybridization and subsequent analysis are completed within 2 hours. Replicate hybridizations on 24 identical arrays with two complex biological samples revealed a mean coefficient of variation (CV) of 11.6%. This study shows the potential of flow-through porous microarrays for the rapid analysis of gene expression profiles in clinical applications.

High Throughput SNP Genotyping for the Human Haplotype Map. *L. Ziaugra¹, J. Moore¹, H. Nguyen¹, A. Lochner¹, M. Faggart¹, M. Goyette¹, R. Barry¹, E. Stahl¹, M.J. Daly², D. Altshuler^{1,3}, S.B. Gabriel¹.* 1) The Eli and Edythe Broad Institute of Harvard and MIT, Cambridge, MA; 2) Whitehead Institute, Cambridge, MA; 3) Harvard Medical School and Massachusetts General Hospital.

The Broad Institute contribution to production genotyping for the International HapMap Project is focused on chromosomes 4q, 7q, 18p and Y, representing about 9% of the human genome. The initial goal of the Hap Map is to successfully genotype 1 SNP with a minor allele frequency $\geq 5\%$ every 5 kb throughout the genome; this milestone will be met by the end of 2004. As of June 2004, we have successfully genotyped 54,552 SNPs in our allocated regions. In evaluating our success against the goal of the 5kb map, we find that approximately 70% of 5kb bins in our allocated regions are complete. Recent additions to dbSNP will be used to help meet our goal. The genotyping group at the Broad Institute has successfully implemented two production scale genotyping platforms to generate data for the Hap Map project: the Sequenom MassArray platform and the Illumina BeadLab. In total we have generated over 7 million genotypes for the Hap Map, about 60% through Illumina and the remainder on Sequenom. Our conversion rate for SNPs on Sequenom is 83%, with a call rate of 99.3%; on Illumina it is 86% and 99.5%. We find similar estimates of genotyping accuracy on the two platforms (99.3% - 99.8%) as assessed by rates of segregation errors and discrepancies in duplicate genotyping. In addition we have completed two externally reviewed Hap Map QA/QC exercises in which data is compared across 10 different Hap Map genotyping centers. In an exercise to assess SNP conversion rate on a random collection of SNPs and genotyping accuracy as compared to a true consensus genotype, the conversion rate for the Sequenom platform was 77% and the accuracy was 99.5%, comparable to other groups. Results of the second exercise to assess accuracy of genotypes already submitted to Hap Map database are pending. Overall we have demonstrated that both platforms can achieve success in terms production data generation and high quality for large-scale genotyping projects. Hap Map data can be obtained via <http://www.hapmap.org>.

Multiple copies of genes are located at segmental duplications that flank the 8p23 inversion variant [inv(8)(p23)] on human chromosome 8p23.1. *N. BOSCH*¹, *Ll. ARMENGOL*¹, *X. ESTIVILL*^{1,2}. 1) GENES AND DISEASE, CENTER OF GENOMIC REGULATION, BARCELONA, BARCELONA, Spain; 2) Experimental and Health Sciences Department, UPF, PRBB, Barcelona, Catalonia.

Genomic architecture of regions containing segmental duplications is of primary importance for the understanding of genomic changes associated with clinical alterations. The genomic organization of the human chromosome 8p23.1 is of special interest due to the presence of clusters of low copy repeats flanking this region. This organization increases the vulnerability of the region to suffer different rearrangements such as the polymorphic inversion found in 26% of the general population, among other rare chromosomal rearrangements. An in-silico analysis of the 8p23.1 region (6Mb in build34) has been performed using Repeatmasker and Pipmaker. This two software packages are a powerful tool to recognize sequences present in more than one copy. Among other multiple-copy genes, we identified 24 copies of a gene coding for the hypothetical protein Q8NF61, from which only five copies are found in the current annotation. On the most proximal part of 8p23.1, twenty-two copies are distributed in 4 clusters of 6, 5, 8, and 3 copies and other two copies are located on the distal part of 8p23.1. Southern blot and PFGE analysis has been performed in order to assess the existence of copy-number and structural polymorphisms in the general population. The complex organization of the different copies of this gene could play a role in the susceptibility of the 8p23.1 region to suffer rearrangements. Comparative studies of this region between human, mouse and rat genomes have revealed several breaks of synteny between these species, with segmental duplications present at the breakpoint regions of the 8p23.1 rearrangements. Furthermore, these segmental duplications contain a large number of genes suggesting that this genomic region could be under positive evolutionary pressure. These findings strongly support a fundamental role of segmental duplications in the evolution of the architecture of this chromosomal region.

Searching for copy number polymorphisms in recently duplicated regions in the human genome. *L. Feuk, T. Tang, J. Cheung, S.W. Scherer.* Department of Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, Canada.

Our analysis of the most recent build of the human genome sequence shows that ~4.3% of the genome is duplicated (using a 5kb and 90% similarity cut-off). Duplication events are important in the evolution of chromosomes and seem to be an ongoing process in the human genome. The aim of the present study is to investigate recently duplicated genes in the genome to determine to what extent gene copy number polymorphism exist in the human population for these genes. A survey of the literature of known gene copy number variants indicate that many such variants are located in regions of segmental duplication. We have compiled a list of candidate genes based on analysis of segmental duplication regions. All genes located completely within duplicated segments were first generated. These genes were then analyzed for their longest open reading frame and preserved intron-exon structure. A comprehensive list of 250 recently duplicated genes with no frameshift or stop codon mutations and with open reading frames highly similar or identical to the paralogous gene was created. Using paralogous sequence variants (PSVs) it is possible to differentiate between the expression of gene copies. Allelic ratios, as well as DNA quantification methods are used to indicate copy number changes for the regions investigated. To date, 35 genes that are represented as two or more copies in the genome sequence have been investigated. PSVs were tested in 10 individuals as well as in a cell line with uniparental homodisomy. Of the 35 genes, nine did not show any evidence of duplication, and may represent errors in the genome assembly. 20 genes were duplicated in all individuals, and six showed a pattern of variation indicative of copy number polymorphism. Further studies are now being performed to verify the copy number variants and link copy number changes to expression of the genes.

High frequency of mosaicism among neurofibromatosis type 1 (NF1) patients with microdeletions caused by somatic recombination of the JJAZ1 gene. *H. Kehrer-Sawatzki¹, L. Kluwe², C.A. Sandig¹, M. Kohn¹, K. Wimmer³, U. Krammer⁴, A. Peyrl⁵, D.E. Jenne⁶, I. Hansmann⁷, V.F. Mautner².* 1) Human Genetics, University of Ulm, Ulm, Germany; 2) Laboratory for Tumor Biology and Development Disorders, Department of Maxillofacial Surgery, University Hospital Hamburg-Eppendorf, Germany; 3) Department of Medical Biology, Medical University Vienna, Austria; 4) Department of Pediatrics, Medical University Vienna, Austria; 5) St. Anna Childrens Hospital, Vienna, Austria; 6) Department of Neuroimmunology, Max-Planck-Institute of Neurobiology, Martinsried, Germany; 7) Department of Human Genetics and Medical Biology, Halle/Saale, Germany.

Detailed analyses of 20 sporadic patients with NF1 microdeletions revealed an unexpected high frequency of somatic mosaicism (40%). 16 of these deletions were identified by a screen of unselected NF1 patients. None of the 8 mosaic patients exhibited mental retardation and facial dysmorphism usually associated with NF1 microdeletions. Our study demonstrates the importance of a general screening for NF1 deletions regardless of the phenotype. In mosaic cases, the proportion of cells with the deletion ranged from 91-100% in leukocytes but was much lower in buccal smears. Thus the analysis of other tissues than blood is recommended to exclude mosaicism in patients with NF1 microdeletions. Further our study reveals breakpoint heterogeneity. The classical 1.4-Mb deletion was found in 13 patients. These type-I deletions encompass 14 genes and have breakpoints in the NF1 low-copy repeats. We identified a second major deletion type that spans 1.2-Mb and includes 13 genes. This type-II deletion was found in 8 of 21 patients (38%) and is mediated by recombination between the JJAZ1 gene and its pseudogene. Seven of the 8 mosaic deletions are of type-II while only one was of type-I. Therefore, the JJAZ1 gene is a preferred target of mitotic non-allelic homologous recombination. While type-I deletions occur interchromosomally during meiosis, type-II deletions are mediated by mitotic intrachromosomal recombination. Thus NF1 microdeletions acquired during mitosis differ from those occurring in meiosis and are caused by different mechanisms.

Correlation of Gene coding region length with human chromosome. *C.M. Malcom¹, J.E. Paschall², G.J. Wyckoff².*
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In mammals, several characteristics, including breadth and pattern of expression, substitution rates and level of functional constraint, are shared among positionally linked genes (Duret and Mouchiarud 2000; Malcom et al., 2003). One factor that has been overlooked in previous studies, however, is gene length. We sought to examine the relationship between positional linkage and the length of gene coding regions. We began with a set of more than 15,500 human genes for which information on the length of the complete coding sequence was available. We compared the central tendencies and distributions of gene coding region length among chromosomes. Although to our knowledge there is no literature to suggest that gene lengths vary significantly among chromosomes, our analysis showed several significant differences in both mean gene length and distributions of gene lengths. Kolmogorov-Smirnov tests showed that approximately 24% of all pairwise chromosomal comparisons yielded significant differences, demonstrating that gene lengths vary across chromosomes. In an effort to put this finding into the perspective of previous results regarding gene properties across chromosomes, we compared the resulting matrix of pairwise differences to the matrix of differences among chromosomes with regard to evolutionary rates from our prior published work (Malcom et al., 2003). The two matrices showed a positive correlation by the Mantel test (p-value = 0.06). To probe this result further, we examined the number of PFAM (Protein Families, Sanger Institute, UK) domains within genes and the length of domains within genes to determine whether or not these also vary across chromosomes. Our results suggest that domain identity within genes may in part explain the linkage between evolutionary rate and gene length in humans. This suggests that any genome-wide study may need to take into account the underlying genomic terrain when significance of genome-wide results is interpreted; differences in gene length, gene number, and evolutionary rate across chromosomes may complicate straightforward analyses of gene properties.

Constructing a Full-length Gene Map of Human Chromosome 7. *Y. Ren¹, J. Zhang¹, K. Nakabayashi¹, J. MacDonald¹, J. Cheung¹, J. Skaug¹, S.W. Scherer^{1,2}.* 1) Dpt.of genetics, The Hosp. for Sick Children, Toronto, ON; 2) Dpt. of Molecular and Medical Genetics, Univ. of Toronto, ON.

Chromosome 7(Chr.7) accounts for about 5% of the human genome and based on a first-generation annotation it contains near to 158 million base pairs of DNA and 1900 gene units. We have been updating the website <http://www.chr7.org/> regularly by adding manually curated genomic and clinical data to provide both the scientific and medical research communities with the most reliable resource for Chr.7. To generate the most complete gene map of Chr.7, we have been using computer-based annotation in conjunction with extensive laboratory experimentation. Partial genes are, in our definition, transcriptional units with an incomplete ORF (missing the start or stop codon). Annotating partial genes will provide information on candidate genes for positional cloning and genotype-phenotype projects and facilitate many studies of Chr.7. We are currently attempting to obtain additional sequence information for the 51 partial genes that we have determined currently reside on Chr.7 by performing (1) 5- and 3-RACE and RTPCR to obtain longer cDNA, (2) Connecting adjacent annotated structures (eg. EST clusters and predicted genes) by PCR to provide evidence for continuity between them at the transcript level (3) RTPCR to confirm gene structures predicted by comparative DNA sequence analysis. Three examples are listed. First, Mus AK033957 and Mus AK041451 are mRNA sequences that are orthologous to the human PGR14 gene. We mapped putative new human exons by comparing the mouse sequence with the human genomic sequence and amplified the remaining regions of the human PGR14 gene by RTPCR. Second, we connected AK027618 and AK075525 by RTPCR and confirmed that they are derived from the same gene. Third, we combined human_THC1646891 and human_THC1667560 with Y19188 and found that this transcript is a PCLO-related gene. All experimental data is submitted to an annotation track in the Chr.7 database so it is available for public viewing. Once completed gene structures are established the resulting information is submitted to GenBank and the Human Genome Organization Nomenclature group for standardization.

Progress in the HapMap-ENCODE project: a comprehensive dataset to guide analysis for the Hap Map. S.B.

Gabriel¹, J.C. Barrett², R. Barry¹, P. deBakker¹, M. Goyette¹, J. Moore¹, H. Nguyen¹, R. Onofrio¹, D. Richter¹, E. Stahl¹, L. Ziaugra¹, A. Motpetit³, T.J. Hudson³, M.J. Daly², D. Altshuler^{1,4}. 1) The Broad Institute, Cambridge, MA; 2) The Whitehead Institute for Biomedical Research; 3) McGill University; 4) Harvard Medical School and Massachusetts General Hospital.

By the end of 2004, the Haplotype Map of the Human Genome will reach a major milestone - the successful genotyping of one SNP (MAF > 0.05) every 5kb throughout the genome in 270 DNAs from four population samples (CEPH, Yoruba, Japanese and Han Chinese). While this initial product will provide a rich resource to support genetic association studies, a detailed understanding of the relationship between SNP density and efficiency of tag SNP picking requires a gold standard dataset in which complete SNP ascertainment and highly accurate genotyping are performed over large and representative segments of the genome in substantial sample sizes. The HapMap ENCODE project was designed by the HapMap Project to address this need, and we will present our contributions to data generation and analysis of this public resource.

In total, ten ENCODE regions, each 500kb, are being studied. SNPs are being discovered by resequencing 48 of the HapMap samples and all SNPs (in dbSNP or newly discovered) in each region are being genotyped in the full set of 270 HapMap samples. We present data on SNP discovery, genotyping, dbSNP comparison, and LD analysis. Key observations already obtained based on initial data in the CEPH population (at a density of one SNP / 600 bp) include the following. First, dbSNP already contains >75% of common sites (>10%) found by resequencing of 16 individuals. Second, at a SNP density of 1/600 bp, 88% of the genome falls into haplotype blocks (as defined by Gabriel et al, Science, 2002), with an average block size containing 41 SNPs and spanning 22kb. We use this dense data to evaluate the performance of the initial HapMap spacing of 5kb, and show that an updated plan for a genome-wide HapMap at 1/kb density will increase the efficiency and power of tag SNPs selected from HapMap.

Different Algorithms Predict Similar Haplotype Block Structures. *A. Indap*¹, *P. Tonellato*¹, *G. Marth*², *M. Olivier*¹.
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Several computer algorithms have been developed to partition genomic sequence into haplotype blocks. These approaches use different assumptions and it is unclear whether the resulting block patterns are specific to the algorithm used. To date, no comprehensive resequencing data for extended genomic intervals are available for large numbers of independent chromosomes to compare the performance of algorithms. We simulated 1000 50-kb haplotypes via the coalescent and compared the effects of SNP density, minor allele frequency (MAF), and the number of ascertained chromosomes on the performance of two partitioning algorithms, MDBlocks and HapBlock. Individual samples were generated by bootstrapping using different SNP density (all markers, 1kb, 3kb, 5kb, 7kb), MAF (all markers, >1%, >5%, >10%, >15%), and sample size (24, 48, 96 chromosomes). Across all datasets, HapBlock infers a greater number of blocks of smaller size. On average, MDBlocks inferred blocks were 1.6 times larger than HapBlock results and contained 1.7 times the number of SNPs. Across bootstrap samples, 95% of blocks inferred by HapBlock were completely contained in blocks inferred by MDBlocks. All block boundaries inferred by MDBlocks were within 1-3 SNPs of the boundaries inferred by HapBlock. This suggests that HapBlock primarily subdivides blocks inferred by MDBlocks into smaller units, but the overall underlying pattern remains the same. Next, we compared shifts in block boundaries when sampling conditions (SNP density, MAF, number of chromosomes) were altered. Regardless of the algorithm, we identified smaller blocks when SNP density was increased, or SNPs of lower MAF were included. However, the average number of blocks inferred increased for the HapBlock algorithm as more chromosomes were included for analysis, but decreased for MDBlocks. Our results show that block partitioning methods are sensitive to SNP density, MAF, and sample size, but the resulting haplotype block patterns are highly similar for the two algorithms. We will present additional data for other algorithms and larger genomic intervals to assess the congruence of algorithms and sensitivity to sampling parameters.

Large scale SNP-based haplotype analysis of the human chromosome 1p that had identified rheumatoid arthritis susceptible variants in PADI4. *T. Kawaguchi¹, H. Kawakami¹, R. Yamada², A. Sekine³, K. Yamamoto², Y.*

Nakamura⁴, T. Tsunoda¹. 1) Lab. Medical Informatics, SNP Research Center (SRC), The Institute of Physical and Chemical Research(RIKEN), Yokohama, Kanagawa, Japan; 2) Lab. Rheumatic Diseases, SRC; 3) Lab. Genotyping, SRC; 4) Res. Gr. Personalized Medicine, SRC.

Case control linkage disequilibrium mapping is one of strongest strategies for common disease-associated gene discoveries. Last year we successfully identified a rheumatoid arthritis (RA)-associated variants of PADI4 in human chromosome 1p36 as a part of large scale survey of whole genome with 70442 SNPs in or close to gene-encoding regions selected from JSNP database (<http://snp.ims.u-tokyo.ac.jp/>). Here we characterize LD and haplotype of 1p. Chromosome 1p spans 120Mb (>3.8% of whole genome) and contains 1125 genes (>5.1% of total genes). 50Mb of the region (41%) encodes gene. We genotyped a total of 93 RA affected Japanese for 3766 SNPs in the region. These SNPs reside on 648 genes, thereby encompassing 58% of currently identified genes in this region. Average density of SNPs was 0.03 SNP per 1Kb in total and 0.06 SNP per 1Kb for 39Mb of gene structured regions. Pairwise LD index was calculated and identified about 270 blocks (35Mb; 30% of the region) with average length being 130Kb. About 20Mb (almost 60% of the whole blocks) of the block segments correspond to gene-encoding region, and about 40% of the gene-encoding regions was within blocks. We applied SNPHAP to phase haplotypes for the LD blocks and haplotypes were successfully inferred for all the LD-blocks. Each LD-block consisted average of 8 common (>1%) haplotypes. Haplotype-tagging SNPs were identified for LD-blocks, and the total number of tagging SNPs was about 1200 (more than 30% of SNPs in blocks).

By applying case-control association tests with these SNPs, disease-associated SNPs were identified and further biological evaluation supported the finding. Identification of the association between PADI4 haplotypes and RA could validate the large scale LD mapping strategy as one of useful tools for the identification of common genetic disorders. TK and HK contributed equally to this work.

Interethnic variability of ERCC2 polymorphisms. *C.R. King¹, J. Yu^{1,2}, R. Freimuth¹, H. McLeod^{1,2}, S. Marsh^{1,2}.* 1) Departments of Medicine, Genetics, and Molecular Biology and Pharmacology, Washington University School of Medicine, Saint Louis, Missouri 63110; 2) Siteman Cancer Center, Washington University School of Medicine, Saint Louis, Missouri 63110.

ERCC2 plays an important role in DNA repair by eliminating bulky DNA adducts produced by platinum agents during the Nucleotide Excision Repair pathway. Several studies have associated polymorphisms in ERCC2 with response to platinum therapy, lung cancer risk, and DNA repair capacity. We examined polymorphisms and haplotype structure of ERCC2 in 95 European, 95 African, and 95 Asian individuals. SNPs (ERCC2 -9164 AT, -1989 AG, -516 GA, 468 CA [Arg156Arg], 1737 CT [Val579Val], 2133 CT [Asp711Asp], and 2251 TG [Lys751Gln]) were mined and mapped using Golden Path, PolyMAPr, and Promolign. Genotyping was performed using PCR and Pyrosequencing. Of seven SNPs identified, the synonymous cSNP at codon 579 could not be confirmed in our populations. There was a clear difference in variant allele frequencies between populations. Variant allele frequencies ranged from 0 to 0.47 in the European population, 0.05 to 0.72 in the African population, and 0 to 0.47 in the Asian population. There were also significant differences in haplotype structure between populations. Of 18 haplotypes, four were common to all three populations. In Europeans and Asians, the most common haplotype was found at 43% and 47%, respectively, while at 0% in Africans. The most frequent haplotype in Africans (53%), was found at 5% and 0% in Europeans and Asians. Our results highlight the interethnic variability of allele and haplotype frequencies in ERCC2. A more comprehensive analysis of ERCC2 would help to clarify the role of polymorphisms with lung cancer risk, DNA repair, and chemotherapy response. This work was supported by the NIH Pharmacogenetics Research Network Grant U01 GM63340.

Fine Mapping and Positional Cloning of Asthma-Related Loci on 5p. *T. Kurz¹, S. Hoffjan¹, G. Hayes¹, N. Cox², K. Deichmann³, C. Ober¹.* 1) Department of Human Genetics, University of Chicago; 2) Department of Human Genetics, Medicine, University of Chicago; 3) Department of Pediatrics, University of Freiburg.

Asthma is a complex phenotype influenced by multiple genes and environmental factors. To identify susceptibility loci, we conducted a genome-wide screen for asthma and related phenotypes in the Hutterites. Markers on 5p13 showed evidence for linkage to bronchial hyperresponsiveness (BHR) with the marker D5S1470 (likelihood ratio (LR) χ^2 $p = 0.0019$) in the Hutterites. At this locus, there was also overtransmission of the 177 bp allele ($p = 0.0061$). Fine mapping of this region was conducted in a sample of 693 Hutterites by typing 75 SNPs in 23 genes across a 31 cM region that included D5S1470. SNPs in 2 genes close to D5S1470 (NPR, ADAMTS12) were associated with BHR ($p = 0.041$ - 0.0037) and SNPs in 3 more proximal genes (PRLR, LIFR, PTGER4) were associated with atopy ($p = 0.042$ - 0.00017). Only the associated alleles in NPR and ADAMTS12 were on the same haplotype as the 177 bp allele. To determine whether SNPs in 1 or more of the associated genes explained the evidence for linkage with D5S1470, we stratified our sample by the presence of the associated 177 bp allele at D5S1470 and each of the 3 proximal loci. The LR χ^2 was reduced from 9.65 to 1.25 when individuals with the associated alleles at both LIFR and PTGER4 were excluded, indicating that nearly all of the linkage was explained by SNPs in LIFR and PTGER4. To replicate these results, we genotyped 56 SNPs in 19 genes in outbred cases (231 asthmatics, 196 atopics) and 270 random controls from Germany. In this sample, SNPs in PTGER4 were associated with asthma ($p = 0.008$) and SNPs in LIFR and NPR were associated with atopy ($p = 0.004$ and 0.04 , respectively). These combined data suggest that this region harbors multiple susceptibility loci, with 1 close to D5S1470 and at least 1 more proximal, that influence asthma, BHR and atopy in the Hutterites and outbred Germans. Supported by HL66533.

Molecular and genealogical analyses of 8765delAG, a BRCA2 founder mutation in high-risk French Canadian families. *H. Vézina², F. Durocher¹, L. Houde², M. Dumont¹, M. Tranchant¹, L. Gobeil², J. Simard¹, INHERIT BRCA_s.*
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The Quebec population contains about 6 million French Canadians who are descendants of the French settlers who colonized Nouvelle-France between 1608 and 1765. These founders account for the major part of the contemporary French Canadian gene pool. The goal of the present study is to gain a better understanding of the role of settlement patterns and demographic history in the spread of the most frequent BRCA2 mutation in our cohort of high-risk French Canadian breast/ovarian cancer families, and to identify the founders who are the most likely to have introduced the mutations. We therefore performed extensive haplotyping analysis of 15 microsatellite markers surrounding the BRCA2 locus and spanning 9 cM on chromosome 13, in 76 carriers of the 8765delAG mutation from 26 families. A highly conserved haplotype is observed for 8 of the 15 markers genotyped. However, in 9 families, among the five most telomeric markers three are different and could thus represent another haplotype. Ascending genealogies of one carrier individual per family (n=26) and of controls (n= 78) were reconstructed using the BALSAC-RETRO genealogical database. These genealogies have an average depth of 11 generations. A number of 15 068 distinct ancestors were identified in the carriers group. Among these, 19 ancestral clusters showed an elevated higher probability of including the founder couple. However, unlike the BRCA1 founder mutation R1443X, for which the founder couple with the highest probability of having introduced the mutation in the Quebec population was identified, no such couple seems to emerge from genealogical analyses, suggesting that either the 8765delAG mutation was introduced more than once, by two separate founders, or that there was a single introduction followed by a recombination event, leading to two different haplotypes. Analyses are currently underway to verify these two hypotheses.

Molecular haplotyping by linking, emulsion PCR: Analysis of PON1 haplotypes and phenotypes. *J.G. Wetmur, M. Kumar, L. Zhang, C. Palomeque, S. Wallenstein, J. Chen.* Box 1124, Mount Sinai School of Medicine, New York, NY.

Linking PCR and emulsion PCR have been combined to create minichromosomes preserving haplotype content. Genomic DNA was dispersed in an emulsion such that less than one template would be found in any aqueous-phase droplet. Four primers were included to amplify separately two linked polymorphic loci. The external primers were present in excess. The internal primers were partially overlapping in sequence and contained 5 biotins. After breaking the emulsion, unlinked duplexes were removed easily on streptavidin-coated magnetic beads. Because the external primers were present in excess, single-stranded unlinked extension products were present that were still capable of subsequent and undesired linking PCR. These contaminants were capped by primer extension on overlapping, complementary 3-phosphate-blocked oligonucleotides. Haplotypes of the minichromosomes were determined by allele-specific real-time PCR. This procedure is robust and requires no additional expensive instrumentation. Human paraoxonase-1 [PON1] is an HDL-associated enzyme with several polymorphisms common to all ethnicities. Promoter polymorphisms are known to affect transcription. L55M may affect PON1 lifetime. Q192R affects substrate specificity. In a population of 390 women of mixed ethnicities for whom we have previously determined genotypes, we determined molecular haplotypes for the double heterozygotes for -909/ L55M (N=95), -909/Q192R (N=80) and L55M/Q192R (N=95). PON1 enzymatic activities were determined in plasma using phenylacetate as well as paraoxon as substrates. The ratio of the activity with these two substrates depends strongly on the Q192R polymorphism. We observed a strong dependence of this ratio on the -909/Q192R haplotype, reflecting increased relative transcription of either Q192 or R192 depending on the linked promoter polymorphism. These results demonstrate the power of molecular haplotype analysis for relating phenotype to genotype. This work was supported by grants from the NIEHS.

A Gene Entrapment Strategy that Generates Homozygous Mutations In Vitro. *S.L. Donahue, H.E. Ruley.*
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The worldwide effort aimed at sequencing the human and mouse genomes is nearly complete, however sequence data alone has not yielded significant insight into identifying gene functions or the relevance of genetic mutation to disease. Thus, practical, genome-wide strategies to characterize gene functions have been developed. Gene entrapment strategies have been used to disrupt genes in murine embryonic stem cells to generate loss-of-function mutations for studies in whole animal models. The primary constraint of this technology, however, is that only one allele is interrupted upon retroviral vector insertion into a random genomic locus. Homozygous gene interruptions are obtained from breeding mice that possess germline heterozygous vector insertions, an undertaking that is ultimately prohibitive for analysis of whole libraries of gene trap cells. Additionally, this issue has restricted the use of gene entrapment technologies in somatic cells. To circumvent this problem, we hypothesized that exposing cells to agents that induce genetic recombination could promote the conversion of heterozygous mutations to homozygous mutations. Several agents that induce homologous recombination were analyzed for their ability to induce loss of heterozygosity (LOH) at the site of retroviral vector insertion in multiple clones obtained from a mouse embryonic stem cell library. While several agents increased LOH at the site of retroviral vector insertion, N-methyl-N-nitrosourea (MNU) was found to increase the rate of LOH by 80-115-fold in all clones examined. Southern blot analysis confirmed that the resulting cells had homozygous mutations at the site of retroviral vector insertion. Furthermore, functional assays performed on a clone with heterozygous interruption of *Xrcc5* showed that the gene product was absent in homozygous mutant cells following MNU treatment. This data shows that this is a powerful tool that can be used to create homozygous mutations in vitro. Additionally, the high rate of conversion and feasibility of this approach suggests that it could be applied to somatic cells to identify genes responsible for various cellular phenotypes.

Effect of menopause on gene expression profiles of circulating monocytes. V. Dvornyk¹, Y.Z. Liu¹, Y. Lu¹, H. Shen¹, J. Wilde¹, T. Conway¹, R.R. Recker¹, H.W. Deng^{1,2}. 1) Osteoporosis Research Ctr, Creighton University, Omaha, NE; 2) Hunan Normal University, China.

Natural menopause is the most important physiological event in female life. It influences general health and functions of various organs and tissues due to profound biochemical and metabolic changes, which are established risk factors. Circulating monocytes are a key element of the immune system. They are major target cells for systemic effect of hormones and actively participate in tissue remodeling by serving as the precursors of various cell types and/or producing cytokines. We analyzed gene expression of circulating monocytes using Affymetrix HG-U133A GeneChip, containing probes for 14,500 genes. The monocytes were isolated from whole blood of 15 women aged 47-55 (7 pre- and 8 postmenopausal). We identified 47 differentially expressed genes ($p < 0.001$). They belong to the various functional categories, including cell signaling and signal transduction (9 genes), transcription regulation (2), apoptosis (1), immune response (4), and others (31). Most of them (36) were downregulated in postmenopausal women. Some of these downregulated genes may be of potential importance for postmenopausal health problems. For example, the EXT1 gene is important for bone metabolism and has putative tumor suppression function. Another gene is FKSG2, an inhibitor of apoptosis. It may contribute to the increased number of monocytes as compared to the premenopausal women, and, subsequently, elevated number of osteoclasts. The placental growth factor gene (PGF) belongs to the family of VEGF-related proteins and may be related to the cardiovascular problems after menopause. STAT1 mediates the expression of a variety of genes, which is thought to be important for cell viability in response to different cell stimuli and pathogens. Among the upregulated genes, SCA7 has been recognized as contributing to retinal degeneration. Another gene of interest is CXorf12, which is located in X chromosome. Its function is unknown. This is the first *in vivo* microarray study of menopause in humans. It demonstrates the prospects of microarrays in genetic dissection of menopause and menopause-associated disorders.

Novel BXSB lupus-susceptibility candidate genes identified by microarray analysis of overlapping chromosome 1 congenic mice. *M.E.K. Haywood, S.J. Rose, M.J. Lees, B.J. Morley.* Rheumatology Section, Eric Bywaters Centre, Faculty of Medicine, Imperial College, Hammersmith Campus, Du Cane Road, London, W12 0NN, United Kingdom.

The BXSB mouse strain is an important model of the glomerulonephritis seen in Systemic Lupus Erythematosus (SLE). Linkage studies have identified several disease-susceptibility intervals, however, the identity of the genes in these intervals remains unknown. Congenic mice represent a defined genetic resource in which the disease-associated interval is bred onto a non-disease prone background strain. In order to identify the lupus-susceptibility genes, we have performed microarray analysis using a series of chromosome 1 congenic lines with partially overlapping intervals. These mice develop specific and limited aspects of the parental disease, permitting phenotypic dissection and the isolation of the contribution of each locus to the overall disease. The genetic difference between each of the lines and C57BL/10, the non-diseased background strain, was less than 5 percent. To ensure that putative disease genes were not arbitrarily rejected, a relatively low fold change in expression levels was studied and the results mapped back to the congenic lines. Simultaneous comparison of the four congenic lines allowed the identification of expression differences associated with specific aspects of disease. Thus we have identified a number of novel SLE candidate susceptibility loci including *Marco*, *Gas5*, *Twist* and *Cd84*, and we have confirmed the identify of *Ifi202* as a disease candidate in the BXSB strain. Sequence analysis of these genes has revealed promoter polymorphisms which may underlie the expression differences that we have confirmed with QRT-PCR. Furthermore, the combination of the microarray results with the different phenotypes of these mice has allowed the identification of a number of expression differences that do not map to a congenic interval, but may be involved in specific disease pathways, notably *Clqb* and *Pawr*.

A comparative genomics approach to SNP-hunting in large genes. *N.S. Wratten¹, J. Simone¹, CH. Chiu¹, L.M. Brzustowicz^{1,2}.* 1) Dept of Genetics, Rutgers U, Piscataway; 2) Dept of Psychiatry, UMDNJ-NJMS, Newark.

Several independent studies have found linkage between chromosome 1q22 and schizophrenia. In this region we found evidence of significant LD between schizophrenia and markers within the *CAPON* gene. A mutation screen of the *CAPON* coding region did not identify any schizophrenia-associated changes in our pedigrees, which indicates that the causative mutation(s) are located in regulatory sequences. The *CAPON* gene extends over 300 kb but only 1.5 kb is exonic and the regulatory sequences are unknown. The large size of *CAPON* poses difficulties common to all association studies of large genes. Firstly, which sequences should be chosen for identification and typing of markers, since a region of this size is expected to contain ~1,500 SNPs? Secondly, how does one overcome the statistical cost of testing multiple SNPs in a moderate-sized sample? Therefore, to facilitate identification of the schizophrenia-associated mutation we took a comparative genomics approach. Regions conserved between primates and rodents are likely to be functional and may have a regulatory role. Targeting these regions for SNP identification has two advantages: The amount of sequence and the number of SNPs to be tested is vastly decreased and those SNPs identified potentially affect function so may increase our chances of testing a causative mutation. Twenty-one conserved sequence blocks were identified, covering 13.8 kb or 3.5% of the entire *CAPON* region. The average block length was 570 bp and each block consisted of at least 10 identical sequences of 15 bp or more. If these blocks are functional we would expect them to be more conserved than other non-coding sequences and indeed comparing verified SNPs in dbSNP there was on average 1 SNP/638 bp and 1 SNP/1057 bp in the entire region and conserved region respectively. We have begun to identify SNPs in the conserved regions for typing and LD analysis. In conclusion, by using comparative genomics we have decreased the size of target sequences more than 10-fold. Our approach should help save time, resources and alleviate problems arising from multiple testing in the identification of disease-associated regulatory mutations.

Self calibration and joint modeling of high throughput genotype data. *S. Ramachandra¹, M. Pratt¹, D. Holden¹, J. Marks¹, A. Spoonde¹, E. Rusman¹, R. Sating¹, A. Diamond², SNPlex Genotyping Program.* 1) Applied Biosystems, Foster City, CA; 2) EnVision Systems LLC, San Diego, CA.

Throughput advances in gene expression and genotyping platforms create qualitatively new challenges in data analysis while at the same time suggesting powerful new approaches to analysis. Typically these platforms operate closer to fundamental limits than their predecessors due to small reaction volumes and higher multiplexy. These factors combined with close physical proximity result in data that have increased susceptibility to covariance effects including varieties of crosstalk and process variability. We present a general approach in which large blocks of data (e.g. plate, run, microarray) are analyzed simultaneously by means of optimal self-calibration. The quantity and inherent redundancy in these data sets allow one to determine much more detailed and accurate parameterization of systematic noise sources than previously possible with serial analyses. The benefit of this analysis is twofold; first, these systematic noise sources can be quantified and tracked, providing much more detailed feedback on instrument and process variables, and second, the systematic variance in the data can be removed producing cleaner data that is less susceptible to error. Application of this methodology to Applied Biosystems high throughput SNPlex genotyping data illustrates these benefits.

SNP genotyping in pooled DNA: an efficient and reliable screening tool for genetic association studies. *L. Wang¹, E. Hauser¹, W. Kraus^{1,2}, C. Haynes¹, J. Rose¹, S. Edgerton¹, L. Huang¹, S. Gregory¹, P. Goldschmidt^{1,2}, J. Vance¹.* 1) Center for Human Genetics; 2) Department of Medicine and Division of Cardiology, Duke University Medical Center, Durham, NC.

Using single nucleotide polymorphism (SNP) at high density for association analyses has been limited by the cost of SNP genotyping for the large number of subjects required. DNA pooling is an affordable alternative. Single base-pair primer extension, followed by denaturing high-performance liquid chromatography (PELC), is a potential tool to genotype SNPs in DNA pools. However, previously it required estimation of a standard correction factor (k) for each SNP, significantly reducing its potential usefulness. We first demonstrated that in association studies k can be eliminated, and then investigated this modified PELC method in an ongoing study on susceptibility genes for coronary artery disease (CAD). Individuals were first grouped based on their CAD index (CAD_i), a validated angiographical measure of the extent of CAD. Groups included 299 young affected (age \leq 55, $CAD_i > 32$), 163 older affected (age $>$ 55, $CAD_i > 74$), and 199 controls (age > 60 , $CAD_i < 23$). DNA pools were made of approximately 100 individuals each, except for older affected. Thirteen SNPs were chosen from a region previously linked to CAD. Modified PELC was used to estimate allele frequency in the DNA pools and each pool was measured three times. To compare with the PELC estimation of allele frequency, each pooled individual was also genotyped using Taqman Allelic Discrimination Assay. In comparison to the allele frequencies obtained via individual genotyping, we found that the average error in using PELC to estimate allele frequency differences between DNA pools was 0.0080.006. In addition, subsequent SNP association analyses, based either on individual genotype data or pooling results were in complete agreement. Using the modified PELC, the reagent cost was approximately 10 cents per SNP per sample under our experimental conditions. We conclude that SNP genotyping in DNA pools using our modified PELC procedure is a cost-efficient and reliable alternative for screening complex diseases for SNP association.

A novel DNA diagnostic method for point-of-care genetic testing: competitive allele-specific short oligonucleotide hybridization with enzyme-linked immunosorbent assay (CASSOH-ELISA). *Y. Matsubara¹, S. Kure¹, M. Hiratsuka², A. Ebisawa², M. Mizugaki², Y. Suzuki¹, Y. Aoki¹.* 1) Dept of Medical Genetics, Tohoku Univ Sch of Medicine; 2) Dept of Clinical Pharmaceutics, Tohoku Pharmaceutical Univ, Sendai, Japan.

With our growing knowledge on the association of genetic polymorphisms and individual variations in drug responses, there has been an increasing promise of personalized medicine. Individualization of drug therapy through genetic testing would maximize effectiveness and minimize risk of medication. In this context, it is important to perform point-of-care genotyping in clinical settings, rather than sending out specimens to a centralized DNA diagnosis laboratory. To date, numerous methods to detect a single nucleotide substitution have been reported. However, these methods require either cumbersome laboratory procedures or high-tech instrument for high-throughput analysis. None of these procedures are readily performed in local clinical laboratories. We previously reported a novel DNA diagnostic method for detecting SNPs using competitive allele-specific short oligonucleotide hybridization (CASSOH) with immunochromatographic strip (*Hum Mutat*, 22:166-172, 2003). The discrimination of a one-base mismatch is achieved by employing unusually short oligonucleotide probes (~10-mer), which would reduce the re-association of mismatched hybrid by a factor of two, providing unsurpassed reliability and reproducibility. We have further devised the method to incorporate enzyme-linked immunosorbent assay (ELISA) for the final detection step, which enabled multiple mutation detection. Starting from 0.5 l of whole blood, genotyping is completed within two hours without sophisticated high-tech equipment or technical expertise. Special ELISA chips have been fabricated so that there is no need for disposing buffer waste, ensuring minimal handling procedures. The method (CASSOH-ELISA) has been successfully applied to the detection of clinically important SNPs in drug metabolism, such as mitochondrial 1555AG, TPMT*3C, and NAT2*5/*6/*7. Our method would facilitate point-of-care genetic testing with potentially diverse clinical applications.

Age-related Alteration of Mitochondrial DNA Copy Number in Leukocytes of Patients with MELAS or MERRF Syndrome. *W. Cheng¹, C. Liu^{1,2,3}, Y. Chen¹, C. Tsai¹, C. Lee⁴, Y. Ma⁴, Y. Chang⁴, B. Wang⁵, Y. Wei⁴.* 1) Vascular and Genomic Research Center, Changhua Christian Hospital, Taiwan; 2) Department of Neurology, Changhua Christian Hospital, Taiwan; 3) Department of Neurology, Chung Shan Medical University and Chung Shan Medical University Hospital, Taiwan; 4) Department of Biochemistry and Center for Cellular and Molecular Biology, National Yang-Ming University, Taiwan; 5) Ctr Medical Genetics, Changhua Christian Hospital, Taiwan.

Mutation of mitochondrial DNA (mtDNA) may affect the copy number of the mitochondrial genome in patients with mitochondrial disease such as MELAS and MERRF syndromes. In this study, we investigated age-related alteration of mtDNA copy number in leukocytes of patients with MERRF or MELAS syndrome. Forty-eight Taiwanese patients including fourteen families with MELAS and seventeen patients including four families with MERRF were recruited. A total of four hundred and eighty-four healthy subjects were enrolled as control. The 4,977 bp deletion and copy number of mtDNA in the leukocyte were determined by PCR. The results showed that the average proportion of 4,977 bp-deleted mtDNA and copy number of mtDNA in leukocytes of patients with MELAS or MERRF syndrome were significantly higher than that of healthy subjects below 30 years of age ($P < 0.05$). By contrast, the average mtDNA copy number in leukocytes was decreased dramatically in patients with MELAS or MERRF syndrome above age 40 ($P < 0.05$). The frequency of occurrence of 4,977 bp deletion and the proportion of mtDNA with A3243G or A8344G mutation in patients with MELAS or MERRF syndrome were found to correlate with the degree of mtDNA depletion in leukocytes. These findings indicate that the mtDNA copy number of leukocytes is increased in early life but is decreased at advanced age of the patients with MELAS or MERRF syndrome. We thus suggest that mtDNA depletion may also occur in affected tissues and play an important role in the pathophysiology and disease progression of the MELAS and MERRF syndromes, respectively, at the later stage of mitochondrial diseases.

Ingenotyping - an highly efficient approach to generate gene targeted mouse and rat models. *J. Laufs, R. Sedlmeier, T. Peters, U. Huffstadt, S. Wattler, M. Nehls.* Ingenium Pharmaceuticals, Martinsried, Bavaria, Germany.

Mouse and rats are the model of choice for *in vivo* studies of gene function but the development of knockouts and allelic variants, i.e. hypo and hypermorphs, is time consuming and costly. The generation of mouse models is dominated by gene targeting through homologous recombination, which requires sophisticated ES cell manipulation and chimera production. No such standard procedure for the development of targeted rat models is available despite some first success in nuclear cloning of rats from unmodified somatic cells. In order to make rat and mouse models with genetic alterations readily available to the scientific community, we developed a patented technology (named INGENOtypingTM) based on gene-saturating chemical mutagenesis. We first applied INGENOtypingTM to generate a pre-set library of mutagenized murine sperm that represents multiple alterations in every mouse gene. Currently somatic DNA and corresponding sperm samples of more than 15,000 mice derived from a well-controlled chemical mutagenesis process using ENU have been archived and can be screened for mutations in any gene of interest within days. Together with the subsequent extremely efficient *in vitro* fertilization, the adult heterozygous carriers are available within 3-4 months. This approach has now been adapted to rats for the industrialized production of gene targeted rat models. ENU mutagenesis in our hands has resulted in more than 220 identified mouse mutations which are to a large extent null but also hypomorphic and hypermorphic alleles. The process and examples of ENU derived mutant mouse lines will be presented.

Differential gene expression analysis in trisomy 21 and normal fibroblasts with the same genetic background. *S. Deutsch*¹, *R. Lyle*¹, *S. Gagos*¹, *M. Moggi*², *C. Baldo*², *S. Dahoun*¹, *B. Bricarelli*², *S.E. Antonarakis*¹. 1) Department of Medical Genetics, University of Geneva, Switzerland; 2) Laboratory of Human Genetics, Galliera Hospital, Genova, Italy.

Individuals with trisomy of chromosome 21 (Hsa 21) or Down syndrome (DS) are affected by a wide range of phenotypes, most of which are only present in a subset of patients. It is not clear how the presence of an extra copy of Hsa 21 leads to DS phenotypes, nor which factors are involved in the phenotypic variability. It is hypothesized that dosage imbalance of Hsa 21 genes either directly or through non-Hsa 21 genes leads to the molecular pathogenesis of this disorder. Genes or pathways involved in DS phenotypes remain to be identified. In order to study the consequences of Hsa 21 trisomy to the transcriptome, and to identify dysregulated genes potentially involved in DS pathogenesis, we have analysed gene expression differences in fibroblasts from normal and trisomic individuals using the Affymetrix technology. A common problem with this type of studies is that unrelated individuals have a large amount of polymorphic gene expression variation not associated to the disease status. To overcome this problem we have adopted 3 different strategies: I) We used cell lines from monozygotic twins, that were discordant for trisomy of Hsa 21. We confirmed the monozygotic status using microsatellites, and performed microarray hybridizations in triplicate. II) We isolated 100% 46, XY clones and 100% 47, XY + 21 clones from an individual mosaic for trisomy 21. Triplicates for each type of clone were used. III) We used fibroblasts from 4 trisomic individuals and 3 normal individuals (unrelated) for microarray analysis. Genes observed to be dysregulated in at least 9 out of 12 possible pairwise comparisons were considered potential candidates. We are currently integrating the data from all experiments using different statistical approaches (GCOS, RMA, transcription module analysis) to identify genes and pathways commonly dysregulated. Our results will give further insights into the molecular consequences of supernumerary chromosomes and possibly suitable candidate genes for involvement in the pathogenesis of DS.

The transcriptome of X-linked recessive myotubular myopathy (XLMTM) muscle. *D. Sanoudou*^{1,3}, *A. Buj-Bello*², *T. Burlison*³, *C. Pierson*³, *J. Laporte*², *J.L. Mandel*², *A.H. Beggs*³. 1) IIBEAA, Athens, Greece; 2) IGBMC, Illkirch, France; 3) Genetics Division, Children's Hospital, Harvard Medical School, Boston, USA.

XLMTM is one of the more severe congenital myopathies. It presents neonatally with profound hypotonia, respiratory distress, major feeding difficulties, absent tendon reflexes and often with dysmorphic features. Pathologically, both fast and slow muscle fibers are affected, with the nucleus centrally located and surrounded by a mitochondrial, glycogen or membranous rich rim. Mutations of the XLMTM gene (MTM1), which encodes a lipid phosphatase called myotubularin, have been found in over 300 families to date.

Using Affymetrix whole genome arrays we analyzed skeletal muscles samples from 7 XLMTM patients versus 7 age-matched unaffected individuals, and 5 Mtm1-knockout (M-KO) versus 5 wild type mice. Data were processed by a combination of bioinformatic approaches including Significance Analysis of Microarrays (thresholds: fold>2 and <5% false discovery rate) and fold change analysis. The most significant changes were consistent across both species including over-expression of 1) cell surface receptor linked- and intracellular- signalling genes, supporting the hypothesis that myotubularin regulates extracellular signaling leading to subsequent effects on muscle function; 2) cell proliferation and muscle development genes, consistent with evidence of muscle regeneration; 3) transcription and phosphate metabolism related genes, pointing to a highly active transcriptional response to MTM1 mutations in skeletal muscle with implications for protein modification. Limited inter-species differences likely represent secondary or species-specific effects of myotubularin mutations, such as over-expression of fibrotic response genes in humans and TGFbeta signalling pathway genes in mice. Despite evidence for aberrant oxidative enzyme activity no significant transcriptional changes were seen for other energy metabolism-related genes.

These data shed light on the molecular pathways of XLMTM development and further validate the M-KO mouse as a representative disease model for future molecular and therapeutic target studies.

In-silico assessment of the impact of single nucleotide polymorphisms in the regulatory regions of human genes.
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Current efforts to identify functional DNA variants were essentially oriented towards single nucleotide polymorphisms (SNPs) found in coding regions of candidate genes since they have direct impact on the structure and function of the affected proteins. Abnormal expression of finely regulated genes could also lead to disequilibria in different metabolic pathways and/or biological processes. Thus investigation of SNPs in the regulatory regions (rSNPs) of candidate genes should improve our knowledge on complex diseases aetiology. We provide here an in-depth in silico characterization of over 1500 rSNPs found in the 2 kb region upstream of the transcription initiation site of more than 170 genes. We assessed the rSNPs density, repartition of the rSNPs over the 2 kb region, inter-SNP distances, as well as the representation of rSNPs in sequence features such as DNA repeats, dinucleotides including CpG islands as well as human-mouse conserved regions. We evaluated the potential of each SNP to disrupt or create putative transcription factors binding sites. Finally we looked at the distribution of rSNPs with respect to the Gene Ontology (GO) classification and found that genes within some certain GO categories had a distinct rSNPs density suggesting that sub-groups of genes might be more or less prone to variability. The putative functional rSNPs are being validated with a combination of approaches including gene reporter and gel mobility shift assays. This in-silico characterization of rSNPs coupled with experimental validation will improve our knowledge on the structure and mode of actions of regulatory regions, and thus provide crucial informations about how to build better predictive models of functional rSNPs. This work is supported by Genome-Quebec/Canada.

Single Nucleotide Polymorphism (SNP) Detection on Microarrays using Ligase Detection Reaction (LDR) and Gold Nanoparticle Reporters. *P. Fortina¹, F. Cao¹, K. Delgrosso¹, P. Patel¹, K. Scott¹, D. Graves², S. Surrey².* 1) Department of Medicine, Thomas Jefferson University, Philadelphia, 19107 PA; 2) Department of Chemical and Biomolecular Engineering, University of Pennsylvania, 19104 Philadelphia, PA.

Microarrays designed for single nucleotide polymorphism (SNP) discrimination usually are based on hybridization of fluorescently-labeled target to probes immobilized on a substrate. Here we demonstrate a novel and sensitive multiplex approach based on barcode microarray technology and gold nanoparticle reporter detection. Reactions involve solution-phase ligation of 2 perfectly templated oligonucleotides, which have different barcode-capture sequences at each end: one to bind a complementary surface-bound probe and the second to bind a complementary solution-phase oligonucleotide target containing a nanoparticle at the other end. In this scenario, one array-bound barcode is needed to capture the normal sequence and a second to capture the mutant or variant sequence. Array capture of either results in a common barcode sequence available for hybridization to a solution-phase oligonucleotide with a bound nanoparticle. Detection then is achieved by silver enhancement resulting in deposition of Ag on the surface of the nanoparticles, enabling scanometric detection to define location of the gold nanoparticles on an array. Attachment of a dye close to the surface of the nanoparticles via linkage to the oligonucleotide also affords detection after silver enhancement by dye fluorescence or by surface-enhanced Raman spectroscopy (SERS). Genomic DNA with known Inflammatory Bowel Disease (IBD) and Haemochromatosis (HFE) gene SNPs were analyzed using this method. Results show the 5 SNPs (3 IBD SNPs and 2 HFE SNPs) were detected successfully using the universal gold nanoparticle detector. By comparing this method with fluorescence-based hybridization, it is anticipated that much higher sensitivity and selectivity can be obtained, indicating that it could be a promising candidate for next-generation biodiagnostic technology.

A Universal Approach to Study Human Disease Genes. *S. Nidtha¹, R. Bennett², K. Poulter¹, R. Fang¹, R. Nutter¹, P. Baybayan¹.* 1) Applied Biosystems, Foster City, CA; 2) Genetics, Children's Hosp, Boston, MA.

Identification of mutations in human genes to determine the genetic basis of diseases is a challenge. With the availability of the human reference sequence, genetic variation in populations is being studied using a combination of methods such as sequencing, dHPLC and SSCP. Each of these methods has advantages, however extensive optimization is required in order to generate accurate data. Sequencing is a preferred method of choice to discover and confirm genetic variation. Researchers who have chosen to sequence their gene of interest have to invest considerable amount of time testing the primers they designed. To eliminate the time consuming step of designing, optimizing and validating PCR primers for human disease genes, Applied Biosystems has developed VariantSEQ[®] Resequencing System. Detecting genetic variations in humans by resequencing has been made simple by this system since it can be easily integrated into any sequencing pipeline. The system takes advantage of the automated capillary electrophoresis platform, as well as reagents and SeqScape v2.1.1 software for mutation detection and report generation. The universal PCR condition and sequencing protocol allow researchers to: 1. Study disease genes with dense mutation spectrum and polygenic diseases 2. Study larger cohorts and therefore conclusions drawn may be more statistically significant. Variations in sequences known or suspected to exist in the human genome can be detected easily and cost effectively. We describe here a new tool for mutation and SNP discovery that is easy to integrate and takes advantage of DNA sequencing, which is a widely accepted.

Comparison of mutation detection in the HEXA gene by dHPLC with endonuclease cleavage. *A. Proos^{1,2}, C. Vaughan^{1,2}, G. Watts^{1,2}, R. Wenzel^{1,2}, C. Jensen^{1,2}, B. Edmonds^{1,2}, L. Burnett^{1,2}.* 1) Laboratory and Community Genetics, Pacific laboratory Medicine Services(PaLMS), Royal North Shore Hospital, St Leonards, Australia; 2) Kolling Institute of Medical Research, Northern Clinical School, University of Sydney, Royal North Shore Hospital, St Leonards, NSW, Australia.

Aim: To compare mutation detection by denaturing high performance liquid chromatography (dPHLC) with the Surveyor endonuclease cleavage method and HPLC detection.

Method: DNA from a pool of enzyme-defined Tay-Sachs disease carrier or inconclusive samples from the Australian population were amplified for each HEXA gene exon. Fragments were hybridised to form heteroduplexes and analysed by

a: dHPLC at optimum melt temperature for each exon;

b: endonuclease cleavage of mismatches followed by sizing by HPLC at 50 deg celsius. Sequence variations detected by atypical or changed peak patterns by each method were confirmed by DNA sequencing.

Results: For each sample the unique peak patterns observed by dHPLC and the calculated fragment sizes after cleavage are shown and discussed. The advantages and disadvantages of each methodology are also discussed.

Evaluation of automated SNP discovery software and its application in the HapMap ENCODE Project. *D.J.*

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Existing software for automated SNP discovery in diploid resequencing data requires manual review to achieve high sensitivity and specificity, which is rate-limiting for large-scale applications. We developed a novel algorithm, PolyDhan, and validated its performance in comparison to and combination with PolyPhred. To determine rigorously and objectively the false positive and false negative rates, we evaluated performance on a large resequencing data set (100 Mb of total sequence: 2.5 Mb in each of 40 individuals) by genotyping all SNPs found, as well as those already in dbSNP, as part of the HapMap ENCODE Project. To our knowledge, no previous evaluation of SNP discovery efforts has integrated SNPs discovered by resequencing with objective verification by genotyping and comparison to dbSNP.

We find that both PolyPhred and PolyDhan perform extremely well on the data set, although each individual program misses some true SNPs, and the lower SNP quality categories in PolyPhred have high rates of false positive calls if not manually reviewed. The combination of the two programs, however, is able to produce very high quality results without human review: we detected over 3,000 novel SNPs (in addition to 2,500 already in dbSNP) with a false positive rate of 7.3%, and, in comparison to confirmed SNPs from dbSNP, a false negative rate of 9.3%.

We also find that 67% of novel SNPs with frequency over 5% are already in dbSNP build 119; that figure rises to 80% for SNPs over 25% frequency. In addition, 89% of novel SNPs showed $r^2 > 0.5$ to an existing SNP in dbSNP build 119. These data underscore the increasing completeness of dbSNP (which now contains 8.8 million SNPs) for choosing a dense collection of SNPs for comprehensive genetic association studies across the genome.

Prevalence of Y chromosome deletions in a Tunisian population of non obstructive azoospermic and oligozoospermic men. *S. ROMDHANE IBALA¹, M. AJINA¹, M. GRIBAA¹, H. ELGHEZAL¹, A. AMOURI², M. ZAOUALI³, A. SAAD¹.* 1) Laboratory of Cytogenetic and Biology of Reproduction, CHU F. HACHED, Sousse, Tunisia; 2) Laboratory of Cytogenetic, Institut Pasteur of Tunis, Tunis, Tunisia; 3) Laboratory of Physiology, Faculty of Medicine, Sousse, Tunisia.

Microdeletions of the long arm of human Y chromosome are associated with spermatogenic failure and have been used to define three regions (AZFa, AZFb and AZFc) that are recurrently deleted in infertile males. Our Objective is to determine the prevalence of microdeletions within the AZFa, AZFb, AZFc subregions in Tunisian patients with idiopathic azospermia or oligozoospermia. One hundred and fifteen patients with idiopathic nonobstructive azospermia (n=58) or oligozoospermia (n=57), have been screened for microdeletion of the long arm of chromosome Y. Those, whose infertility was related to known hereditary, constitutional cytogenetic abnormalities, endocrine or obstructive causes were excluded. Blood and semen samples were obtained from all of the patients. We performed for every one semen analysis. we measured FSH, LH, and testosterone levels and performed karyotype analysis. We looked for microdeletions using 2 multiplex PCR with 7 pairs of primers directed to Y-specific STS genomic markers from the three Y chromosome AZF regions. Overall, 3 of the 115 infertile patients tested exhibited deletion of the Y chromosome, 2 of them being azospermic and 1 extreme oligozoospermic. All patients presented a normal karyotype and deletions were confined to the AZFc region, involving the DAZ locus. In contrast no deletion was detected in AZFa or AZFb. The frequency of Y chromosome microdeletion in our sample (2,6 %) is in the lower limit of the frequencies reported by the literature. This confirms that the frequency may be affected by population structures in different geographical regions. In conclusion we confirm that deletion of the AZFc region of the Y chromosome is the most frequent molecularly defined cause of spermatogenic failure. AZFc deleted men are candidates for ICSI, since in most cases spermatozoa or mature spermatids suitable for the procedure can be recovered from semen or from testis biopsies.

High-throughput Detection of Regulatory Polymorphisms in Candidate Genes Relevant to Common Diseases.

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Understanding genetic variation is critical to reveal the causes to complex diseases and therapeutic responses. Until recently, the emphasis was on the detection of single nucleotide polymorphisms (SNPs) in the coding regions of candidate genes because of their direct effects on the structure and function of the affected proteins. However, transcription factor binding sites lying in the 5 regulatory regions might be disrupted by a SNP leading to changes in expression levels of finely regulated genes. Unfortunately, little is known about the nature and the prevalence of SNPs in the regulatory regions (rSNPs). Thus, investigation in rSNPs of candidate genes should increase our knowledge of the aetiology of complex diseases. This study aimed at identifying the rSNPs in hundreds of candidate genes, selected based on the possible implication of their protein product in cardiovascular diseases, inflammatory disorders, cancers and therapeutic responses. The regulatory regions, arbitrary defined as a 2kb genomic segment upstream of the transcription initiation site, were screened by dHPLC for the presence of SNPs in a population panel of 40 individuals from different ethnic background. In this report, we have screened the regulatory regions of over 190 genes (380 kb of genomics DNA/individual). The nature and position of the rSNPs was confirmed by sequencing. We found the presence of 1554 distinct rSNPs, equally distributed throughout the 2 kb region. Only 20% of these rSNPs have previously been described in public databases such as DBSNP, HGBASE and HGMD. In silico analysis has led to the identification of 77 rSNPs (5%) in conserved regions as well as 1165 rSNPs (75%) affecting predicted binding sites of transcription factors. The functional importance of these rSNPs is being assessed by a combination of methods (gene reporter and gel shift analysis). The detection and validation of rSNPs are relevant to biomedical research by providing a valuable set of targets for discovery of genes related with diseases and therapeutic responses. This work is supported by Genome-Quebec/Canada.

Transcriptional deregulation by adenovirus E1A in human tumor cells: reprogramming of tumor cells to less malignant phenotypes. *J. Dorsman*¹, *A. Teunisse*², *H. Verdurmen*¹, *T. Plug*¹, *D. Rockx*¹, *R. Verheijen*¹, *A. Van der Eb*³.
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The adenovirus 5 (Ad5) E1A protein can reverse the transformed phenotype in a wide range of human tumor cells and can suppress primary human tumor growth. E1A apparently interferes with oncogenic pathways by its ability to reprogram transcription in human tumor cells. To get more insight which genes are deregulated by E1A in human tumor cells, Affymetrix expression array studies were performed analysing a glioblastoma model system. T98G cell lines which stably express the 12S Ad5 E1A gene were compared with cell lines generated by transfection of the same vector but lacking the E1A gene. The E1A-expressing cell lines do not give rise to any colony formation in soft agar, whereas the control cell lines efficiently form colonies under the same conditions. In agreement with previous studies, an up-regulation of genes involved in the heat shock response was observed. In addition, other groups of co-regulated genes are found, including the down-regulation of a group of genes coding for metalloproteases and of genes involved in xenobiotic metabolism. The Affymetrix data were validated with LightCycler PCR. Subsequently, bioinformatic approaches were used to identify transcription factor binding sites enriched in up- or down-regulated promoters respectively. We found, that the down-regulated promoters were enriched for AP1 elements. This finding is in agreement with the already known negative effects of E1A on AP1. Also other elements not previously associated with E1A were found. The implications of these findings are discussed.

A minimization entropy-based algorithm for modeling bipartite motifs with application to PXR/RXR binding sites. *C. Bi^{1,2}, C. Vyhldal¹, J.S. Leeder¹, P.K. Rogan^{1,2}*. 1) Children's Mercy Hospital, Schools of Medicine and; 2) Computer Science and Engineering, University of Missouri-Kansas City.

We developed a new algorithm for discovering bipartite cis-regulatory patterns based on Shannon's entropy minimization principle and applied to a set of known PXR/RXR binding sites. A bipartite module is an independent functional unit on the upstream of a regulated gene and recognized by a protein binding complex such as PXR/RXR heterodimer. We assume that two proteins (PXR and RXR) cooperatively bind to the module with constrained spacers. The heterodimer binding controls the expression of co-regulated genes such as CYP3A4, which is involved in detoxification of drugs and xenobiotics. A bipartite model built to simulate a bipartite module has two components, left and right motifs and the associated gap penalty function. The goal is to maximize the total information content which can be reduced to minimize the total Shannon entropy. We used Monte Carlo strategy to greedily search the multiple alignment space and find an optimal solution to the bipartite pattern problem. The algorithms were implemented in C++/Perl and successfully applied to finding homogeneous and bipartite motifs using a set of PXR/RXR protein binding sites. We built the models for different motif widths and validated them based on the relative binding strength of the testing sequences. Based on the regression models, we computed the correlation coefficients (r) and Akaike information criterion (AIC) for each model. The best model is the one with the smallest AIC values. Among the best bipartite models are 7<3>7 (7 bps on left and right half-sites with dominant spacer of 3 bps), 8<2>7 and 9<2>7 ($r > 0.83$). The results support our hypothesis that PXR and RXR transcription factors cooperatively bind to two adjacent motifs with variable spacing.

CEPH cell lines are representative of the American Caucasian population: implications for pharmacogenetics.

M.A. Meucci, S. Marsh, H.L. McLeod. Molecular Oncology, Washington University, St. Louis, MO.

A better understanding of genetic influences on drug response is needed to improve the identification of patients that will either benefit or be harmed by therapy. If an adequate sample size is collected, the quantity of available DNA may not be adequate for a comprehensive analysis. CEPH cell lines are a collection of immortalized lymphoblastoid cells from multiple generations of large families. They have previously been critical for genome mapping, and have been used by the International Haplotype Mapping project and by SNP consortium. They have been used to identify QTLs associated with transcriptional control, apoptosis after irradiation and recently have been used to identify cytotoxicity after cancer chemotherapy. There is no data on how representative CEPH cell lines are for genetic variants of pharmacogenetic significance. Genotype and allele frequencies of twenty-eight variants in fifteen clinically relevant genes were analyzed in germ-line DNA from 95 European-Americans and 95 Africans, and 80 CEPH cell lines of American Caucasian origin. The results demonstrate that allele frequencies for the twenty-eight polymorphisms are not significantly different ($p > 0.05$), between the CEPH cell lines and the European germ-line DNA. The Spearman rank value for the comparison between the CEPH and European allele frequencies is 0.954 with a 95% CI of 0.899-0.979. We have demonstrated that CEPH cell lines of American Caucasian origin do not significantly differ in allele frequency compared to the European germ-line DNA. The Spearman rank value for the comparison between the CEPH and African allele frequencies is 0.554 with a 95% CI of 0.181-0.787 demonstrating that, as expected, CEPH cell lines of American Caucasian origin will not provide a high level of prediction for African DNA analysis. It can be stated that in research involving a reference population, the relevant population equivalent CEPH cell lines can be substituted as an ideal DNA resource without compromising quality for quantity, providing pharmacogeneticists with a renewable resource for association, population, and functional analysis of clinically relevant polymorphisms.

Physical Mapping of the Proximal p Arm of HC21. *E. Miller¹, L. Chen¹, E. Becka¹, R. Ganith¹, A. Hohmann¹, E. Hood¹, M. Bozovsky¹, C. Holmes¹, M. Cummings², J. Doering¹.* 1) Loyola Univ Chicago; 2) Univ. of Illinois, Chicago.

Centromeric and other heterochromatic regions, which comprise 10-15% of the human genome, are not included in the completed sequence. We are constructing a detailed physical map of the centromere and p arm of HC21 as a model for the organization of such regions. Our previous work found that proximal 21p has a number of low copy number repeat sequences (LCNR) also found in the centromere of HCY. The linear order of these elements on the proximal p arm of HC21 strongly resembles that on HCY. We have sequenced six of the LCNR from HCY (KFC11, KFC37, KFC43, KFC52, 64b, YIII.1). BLAST results indicate that three of these elements (KFC11, KFC37, KFC52) are contained in the human chAB4 duplicon. The chAB4 duplicon is ~86kb long and composed of a chAB4 DNA segment adjacent to a 48bp satellite array of variable length, followed by an NF1-related pseudogene. Work by others suggested that the chAB4 duplicon normally occurs as two adjacent copies with a palindromic organization. However, our mapping studies show that the chAB4 duplicon is not in a palindromic organization on either HC21 or HCY. The organization of the chAB4 duplicon and adjacent LCNR appears to be the same on HC21 and HCY, which clarifies an unsequenced HCY centromeric region. The chAB4 duplicon and adjacent LCNR are located in the 21-II region of the proximal p arm of HC21. This region is composed of a complex array of monomeric alphoid DNA along with SINES, LINES, and other satellite DNA. Comparing the sequences of the duplicon and adjacent LCNR on HCs 21, Y, 22, and 17 indicates copies of these sequences on different chromosomes share at least 90% sequence similarity. It also indicates that chAB4 may be part of an even longer ~200kb duplicon. LCNR corresponding to ~63kb of the chAB4 duplicon are also present on the distal p arm of HC21. A segment of ~13kb of the duplicon is also found in HC21q adjacent to the D21Z1 alphoid array. Thus various portions of the duplicon have apparently moved independently in the genome. LCNR located in the proximal p arm of HC21 will allow construction of a contiguous map of ~5Mb that links 21-II to the centromeric D21Z1, and joins the p arm to the q arm.

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Copy Number Variation in the Human Alpha Defensin Genes *DEFA1* and *DEFA3*. *P.M.R. Aldred, E.J. Hollox, J.A.L. Armour.* Genetics, Univ Nottingham, Nottingham, United Kingdom.

Human defensins are small cationic peptides that form part of the innate immune system, acting against a variety of bacteria, fungi and also some viruses. To date, a cluster of six human alpha defensins has been identified on chromosome 8p23.1. *DEFA1-DEFA4* are expressed in neutrophils and neutrophil defensins appear to have some anti-HIV activity. *DEFA1* and *DEFA3* differ by a single amino acid change and the corresponding genes have been shown to vary in copy number between individuals. Here we use Multiplex Amplifiable Probe Hybridisation (MAPH), Pulsed-Field Gel Electrophoresis, Southern Blotting and ratios from restriction fragment digest assays to investigate the extent of this copy number variation in the general population. The results show that diploid copy number of *DEFA1* can range from three to nine copies with four to six being most common. The diploid copy number of *DEFA3* was found to range between zero and four copies with most individuals having one or two copies. 18/145 samples were found to lack the *DEFA3* gene, corresponding to a previous report that approximately 10% of people lack the *DEFA3* peptide. Current work is moving on to study the relationship between genomic copy number and expression levels of *DEFA1* and *DEFA3* by comparing ratios from restriction digest of cDNA products with those from genomic DNA.

Interleukin-10 -1082 G/A polymorphism and Risk Of Bronchopulmonary Dysplasia (BPD). *R. Baier, J. Loggins, K. Yanamandra.* Dept Pediatrics, LSU HSC, Shreveport, Shreveport, LA.

IL-10 expression is affected by a single nucleotide polymorphism (SNP) located at position -1082 (G to A). The A allele is associated with lower IL-10 production. Low IL-10 production has been linked to the development of BPD. Thus, the IL-10 -1082 SNP may be a genetic risk factor for the development of BPD. The IL-10 -1082 SNP was determined in 294 (235 African-American, 56 Caucasian and 3 Hispanic) mechanically ventilated VLBW infants at risk for BPD. The incidence of the A allele in our population was 0.62. Thirty-nine (13.3%) infants were GG, 146 (49.7%) were GA and 109 (37.0%) were AA. There were no significant differences between genotype groups with respect to ethnic origin, gender or need for surfactant replacement therapy. AA infants were slightly more mature and of greater birth weight than GA infants (27.0 0.2 wks vs. 26.30.2 wks; $p < 0.05$: 94022 gms vs. 88218 gms; $p < 0.05$). There was no significant effect of the IL-10 -1082 SNP on mortality or development of BPD. However, the AA/GA genotypes was associated with a reduction in risk for the combined outcome of BPD or death (18/38 vs. 80/256; $p = 0.049$).

Outcome	GG (n=39)	GA (n=145)	AA (n=110)	p
Early Death	6 (15%)	14 (10%)	8 (7%)	0.340
Total Mortality	8 (21%)	20 (14%)	15 (14%)	0.536
BPD	11 (34%)	30 (23%)	22 (23%)	0.366
Death or BPD	18 (46%)	46 (32%)	34 (31%)	0.189

The IL-10 -1082 G/A SNP may influence mortality and or the development of BPD in ventilated VLBW infants.

(XY)_mX_n repeats are a common, variable, and evenly distributed polymorphism. *T. Hefferon, D. Cutler, G.R. Cutting.* Inst Genet Med, Johns Hopkins, Baltimore, MD.

While studying the *CFTR* gene, we discovered that a variable TG dinucleotide tract immediately adjacent to a variable T mononucleotide tract displayed a unique form of co-variation. Nucleotides at the interface of TG and T repeat tracts were found to be highly susceptible to point mutations, G>T or T>G, that resulted in simultaneous changes in repeat number of both tracts. To determine if other examples of these repeats exhibited similar variation, we scanned the genome for loci that conformed to the pattern (XY)_mX_n, where m ≥ 5 and n ≥ 5 and identified 41,294 loci. On the other hand, we discovered only 2,930 examples of (XY)_mZ_n tracts. We then determined the distribution of (XY)_mX_n repeats across the human genome. The most abundant group, (TA)_mT, contained 15,110 examples with a mean of 5.3 ± 3.0 per megabase, or roughly one per 200 kb. To determine the extent of variation at (XY)_mX_n loci, we sequenced DNA samples from 18 individuals of diverse geographic origin at randomly selected sequences representing all purine/pyrimidine combinations of di- and mononucleotide tracts ((TG)_mT (n=5), (TC)_mT (n=5), (TA)_mT (n=3), (GC)_mG (n=2), and (GA)_mG (n=3)). Evidence of mutation occurring at the interface of the di- and mononucleotide tracts was observed in all groups except (GC)_mG. The extent of polymorphism varied amongst the combinations, and was highest in (TG)_mT and (TC)_mT, each of which contained an average of seven different alleles in our sample of 36 chromosomes. In addition, sequencing of the *CFTR* (TG)_mT locus in twenty-two chimpanzees revealed two variants, which differed from one another by a mutation at the interface of the two tracts. (XY)_mX_n repeats thus represent a unique form of genetic polymorphism that is both widely distributed and highly informative, and which has potential applications to linkage, mapping, and evolutionary studies.

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A significant portion of false single nucleotide polymorphisms may not be revealed by large-scale validation. *M.J. Luo, X.F. Cui, D.M. Frikker, M.A. Azaro, H.Y. Wang, I.V. Tereshchenko, G.H. Hu, H.H. Li.* Dept. of MGM/The Cancer Inst of NJ, UMDNJ, 195 Little Albany St, New Brunswick, NJ 08903.

More than 10 million single nucleotide polymorphism (SNP) submissions have been received by the public databases. Large-scale effort is being made to validate these genetic markers. Our recent study indicates that a significant portion of false SNPs may not be revealed by the commonly used validation methods. In order to evaluate the limitations associated with the conventional validation methods, 2357 SNPs were selected and reevaluated with three different new approaches, gel electrophoresis, single-sperm test and population analysis. It was found that 4.4 % of these SNPs were not real and may be attributed to paralogous sequence variants. Among the 4.4% false SNPs, 30% may not be revealed by the commonly used validation methods. We also showed that the accuracy of genotyping method could be limited by unknown polymorphisms in the primer or probe regions. Approximate 0.35% of typing errors can be attributed to this problem. The present study indicates that no method alone could identify all false SNPs and SNPs in the databases should be used with caution even if those have been validated. Some of the measurements on SNPs quality present in the database, such as DoubleHit, may not be very meaningful. Gel electrophoresis after PCR amplification is an efficient method for primary and rapid screening. For a high level of accuracy in SNP selection, combined methods should be used.

Functional polymorphisms in the putative promoter region of the human equilibrative nucleoside transporter 1 gene. *S.N. Myers*^{1,3}, *J.D. Roy*^{2,3}, *R.K. Goyal*^{1,3}, *R.E. Ferrell*^{2,3}. 1) Pediatric Hematology/Oncology & Bone Marrow Transplantation, Children's Hosp of Pittsburgh; 2) Department of Human Genetics; 3) University of Pittsburgh, Pittsburgh, PA.

Induction chemotherapy regimens containing cytosine arabinoside (ara-C) and anthracyclines result in 80% complete remission rate in childhood acute myeloid leukemia (AML) but their cure rate is about 35 - 50%, one of the lowest of all childhood cancers. Understanding the factors that contribute to emergence of chemoresistant leukemic cells is crucial to improving treatment outcome in children with AML. Ara-C is the most active anti-metabolite for inducing remissions in AML. Human equilibrative nucleoside transporter 1 gene (hENT1) may play a significant role in determining outcome of AML therapy because it transports Ara-C into leukemic blast cells and is down-regulated in resistant blast cells. We resequenced 1.6 Kb of the 5'-flanking region of hENT1 (SLC29A1) in 48 unrelated individuals and identified four haplotypes comprised of single nucleotide polymorphisms (SNPs) at positions -1345C/G, -1050G/A, and -706G/C. TRANSFAC analysis predicted that -706G/C would alter consensus transcription factor binding site sequences. Expression of reporter gene luciferase was analyzed in the COS-1 cell line transfected with the luciferase vector pGL3-BASIC containing the 1621 bp region spanning from -2 to -1622 of the hENT1 upstream sequence with the wild type CGG sequence, or one of the CAG, GAG, or CGC variants. The expression of luciferase increased by 105% in the CAG ($p < .0001$) and 52% in the CGC variant ($p < .0001$), as compared to the CGG wild type haplotype. A 5% increase from the CGC haplotype was not significant. These data suggest that the CAG and CGC haplotypes of the hENT1 upstream region may influence hENT1 expression and be functionally relevant. We are currently genotyping a cohort of 94 Caucasian individuals of mixed European ancestry at these loci for haplotype frequency distribution. Additional experiments are underway to test the significance of this variation in hENT1 gene in a large group of children treated on ara-C based cooperative clinical trials.

Comparison of whole genome amplification methodologies and sources of biological sample for high-throughput SNP genotyping. *J.W. Park*^{1,2}, *T.H. Beaty*¹, *P. Boyce*³, *A.F. Scott*^{2,3}, *I. McIntosh*². 1) Epidemiology, Johns Hopkins University, Baltimore, MD; 2) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins School of Medicine, Baltimore, MD; 3) Center for Inherited Disease Research, Baltimore, MD.

The amount of genomic DNA required for extensive genetic analyses may exceed that available, especially for stored samples. Development of methods for whole genome amplification (WGA) circumvents this problem. Two such methods, multiple displacement amplification (MDA) and OmniPlexTM technology, were compared to determine the efficiency and accuracy of amplification measured by SNP genotyping using the Bead Array method on 1,260 markers in 18 unrelated individuals. The success rate of genotyping from amplified DNA by MDA (GenomiPhiTM, Amersham) was lower than that from un-amplified DNA (90% vs. 98.9%) but higher than any other WGA method (64.1%~88.6% for OmniPlexTM with or without 7-deaza-GTP, & TempliPhiTM). The number of mismatches between pre-amplified and amplified DNA extracted from whole blood was lowest for GenomiPhiTM and highest for OmniPlexTM with 7-deaza-GTP as an additive (0.21% vs. 1.33%, $p < 0.01$) among four different WGA methods. The efficiency and accuracy of MDA was also compared on a refined 1,228 SNP panel using different sources of genomic DNA (whole blood, buccal swab, and dried blood spot) from the same individuals. Amplification of DNA from buccal swabs was both less efficient and less accurate than from whole blood (97.6% vs. 99.5% and 0.35% vs. 0.06%, respectively) but superior to DNA from dried blood spots despite the use of 3 independent extraction methods (31.2%-90.3% success rate, 14.5-44.9% mismatches). The low efficiency and accuracy of amplification when using DNA extracted from dried blood spots as template may be due to its low molecular weight, since sheared genomic DNA (< 20kb in length) also gave high rates of SNP genotype mismatches and low efficiency. In conclusion, MDA represents an efficient method to maximize DNA resources from whole blood and buccal swabs.

A simple PCR-RFLP method to type the G1162A renin gene SNP (formerly *BgII*) and its frequency in a Mexican population. *L.L. Valdez*^{1,2}, *F. Mendoza-Carrera*^{1,2}, *H. Montoya-Fuentes*¹, *F. Rivas Jr.*³, *F. Rivas*¹. 1) Western Biomedical Research Center, Instituto Mexicano del Seguro Social; 2) Graduate Studies Program in Human Genetics, Universidad de Guadalajara, Guadalajara, Jalisco; 3) School of Medicine, Universidad de Colima, Colima, Mexico.

Renin is the main rate-limiting enzyme in the renin-angiotensin system (RAS) cascade that leads to the production of angiotensin II. The enzyme and its gene (REN), located at 1q32, are candidate underlying factors in essential hypertension and cardiovascular disease. *BgII*, one of the most commonly investigated REN polymorphisms, has been studied either by Southern-blot or PCR. Previously published PCR techniques involved long amplicons (3.9 kb) or used less-than-ideal primers, and misidentified the precise SNP location. In this report, a new PCR procedure to type REN *BgII* polymorphism is presented. Newly designed primers: 5' ATAATAACAGTACCTACTTGATG 3' (F), 5' CTACAGTCTTAATGAATCACTT 3' (R) were used. Acrylamide electrophoresis disclosed either a single- (allele A, 537 bp) or two-band (allele G, 371+166 bp) pattern per chromosome after *BgII* digestion. Sequencing demonstrated this polymorphism is located at nucleotide position 1162. DNA samples from 129 non-related volunteers were typed following the present PCR-*BgII* protocol. This group of healthy mestizo adults living in Guadalajara is an admixed population with 56% Spanish component. Allele frequency was 0.30 G and 0.70 A, and genotype frequencies 15/129 (11.6%) G/G, 48/129 (37.2%) G/A, and 66/129 (51.2%) A/A. Frequencies are in agreement with Hardy Weinberg expectations ($\chi^2=1.79$, 1 df, $p>0.05$). Allele distribution in this study is similar to that observed in Caucasian populations (range 30-39%). Since this RFLP shows a worldwide diversity (6 to 48% allele G), population-specific data for human REN SNP G1162A must be considered to study RAS in normal and diseased groups. The method here presented is reliable, simpler, and less expensive than previously developed ones.

Cost-benefit and high-throughput method for polymorphism validation. *H.-C. Yang¹, C.-H. Lin², C.S.J. Fann¹.* 1) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; 2) Institute of Genetics, Yang-Ming University, Taipei, Taiwan.

Using dense SNP markers to locate disease susceptibility genes of complex disorders becomes a mainstream of fine mapping. However, only polymorphic SNP markers provide statistical power to assess LD. The public-domain genetic databases only provide partial information of polymorphism for populations of various ethnic backgrounds. Hence, validation of polymorphism for the studied ethnic group is always essential prior to initializing a large-scale genotyping experiment.

In this paper, we employ DNA-pooling approach based on the platform, MALDI-TOF mass spectrometry (Sequenom), to achieve rapid and economic polymorphism validation. Variation inherent in DNA pools is controlled by replications. To evaluate the performance of the proposed strategy, we carry out an experiment with 185 SNPs located in HLA region of chromosome 6. The SNP information is published in Walsh et al. (2003). Based on our previously collected normal samples consisting of 3400 individuals residing in Taiwan, 630 individuals are selected randomly and their genomic DNA is pooled together. For each SNP, sixteen observations from four replicates for each combination of two PCR amplification and two chips are analyzed. On the other hand, 95 individuals are also chosen randomly from the normal samples and genotyped individually for confirmation purpose.

Firstly, we compare the allele frequencies from our individual genotyping experiment and Walsh et al's experiment which is contributed by European-descent CEPH families. The discrepancy of different ethnic groups reflects on the inconsistent patterns of polymorphism. Twenty-one SNPs out of 185 SNPs cannot be validated suggests a misclassification rate of 11.35% for the two ethnic groups. Secondly, based on our proposed method, we find high consistency in terms of polymorphism validation between our DNA-pooling and individual genotyping studies. The sensitivity is 93.29% and specificity is 100%. These results suggest that using DNA pooling is cost-benefit and information-retained compared with individual genotyping in polymorphism validation.

A comparative study of the neighboring-nucleotide biases on single nucleotide polymorphisms in the mouse and human genomes. *F. Zhang*¹, *Z. Zhao*^{1,2}. 1) Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University, Richmond, VA; 2) Center for the Study of Biological Complexity, Virginia Commonwealth University, Richmond, VA.

A comprehensive understanding of single nucleotide polymorphisms (SNPs) in the human, mouse and other model organisms is essential to unravel the genetic basis of phenotypes, functions, and diseases. The substitution patterns at polymorphic sites and the sequence context in a local environment of SNPs reflect the mutability of the sequence and, therefore, are important for studying the molecular mechanisms of the mutation and the genome sequence evolution. In this study, we first analyzed the neighboring-nucleotide composition of 469,455 biallelic SNPs in the mouse genome and then compared the results obtained from 8,043,656 human SNPs. These data were obtained from NCBI's dbSNP database and represented the largest public collection of SNPs at present (dbSNP build 121, released in June 2004). As in humans, large neighboring-nucleotide biases relative to the genome- or chromosome-specific average were observed in mice at the immediate adjacent sites. For all substitutions, the biases for A, C, G, and T were 0.12%, 2.90%, 0.69%, -3.71%, respectively, at the immediate adjacent 5' site and -3.87%, 0.75%, 2.98%, 0.14%, respectively, at the immediate adjacent 3' side. The biases decreased by increasing distance from the substitution site. An examination of the six categories of substitution revealed the different patterns for transitions and transversions. For transitional substitutions, we observed the large biases on nucleotide C at the nearest 5' site and G at the nearest 3' site, reflecting the strong influence of the hypermutability of dinucleotide CpG in the mouse genome. The patterns of neighboring effects on transversional substitutions were complex. In both genomes, the probability of a transversion increased with increasing A+T content of the two immediate adjacent sites. In conclusion, the bias patterns for the neighboring nucleotides in the mouse and human genomes were essentially the same; however, the extent of the biases was remarkably less in mice.

Analyzing individual BioGeographical ancestry and admixture structure in human populations. *I. Halder¹, R. Mei², E. Parra³, J. Akey⁴, M. Shriver¹*. 1) Dept Anthropology, Pennsylvania State Univ, State College, PA; 2) Affymetrix Inc. Santa Clara, CA; 3) University of Toronto at Mississauga, Mississauga, Canada; 4) Fred Hutchinson Cancer Research Center, Seattle, WA.

Understanding genetic variation in populations and how this variation contributes to differences in disease risk among populations is an important area of research. We have typed a panel of 11,555 SNP markers in three resident US populations, (African Americans, Puerto Ricans and European Americans) in an effort to analyze patterns of genetic variability. The first two are considered admixed populations with varying levels of BioGeographical ancestry (BGA), while the European Americans are assumed to be more homogeneous. Putative ancestral populations were chosen from among a panel of twelve world populations typed for the same markers. Using a maximum likelihood based method and a separate Bayesian method we show that individual BGA estimates vary within all groups. Assuming a trihybrid model of admixture between Europeans, West Africans and Native Americans we observed large variations in individual BGA levels in both the African-American and Puerto Rican samples, while the European Americans are more tightly clustered. Additional simulation studies were done to check the reliability of the estimates under the specified model. Simulations further indicate that the choice of ancestral population and its sample size both affect individual BGA estimates.

To test for stratification within the population samples we have used the Individual Ancestry Correlation (IAC) test, where markers are split into two non-overlapping subsets and ancestry is estimated separately with both sets. Correlation between estimates obtained with the different marker sets indicates presence of genetic structure. While both the African-American and the Puerto Rican samples show admixture stratification as expected, we also detected evidence for stratification within the European-American sample. Additional simulations showed that individual BGA estimates differed when only a subset of informative markers was used as opposed to including all markers.

In vivo analysis of p53 protein binding to consensus sites in the p21, GADD45, MDM2, XPC and XPE genes in normal fibroblasts irradiated with ionizing radiation

p21GADD45MDM2XPCXPE. W. Dridi¹, R. Fetni², R. Drouin¹. 1) Dept Pediatrics, CHUS, Quebec, QC, Canada; 2) Cytogenetics, Montreal Children Hospital, Montreal, QC, Canada.

Introduction: As a transcription factor, p53 protein controls the expression of more than 30 genes. P21, GADD45, MDM2, XPC and XPE genes are amongst the most downstream effectors of p53. In the literature, in vitro analysis gives strong evidence that p53 binds to its consensus sequence in these genes. However, only one study has shown in vivo the interaction of p53 with p21 and GADD45 genes in human myeloblastic leukemia cell line (ML-1). **Objective:** To analyze p53 binding in vivo to its consensus sequence on p21, GADD45, MDM2, XPC and XPE genes in normal human fibroblasts following gamma irradiation. **Methods:** Human fibroblasts were exposed to 20 Grays of gamma irradiation. At different time points (0, 1, 2, 4 and 8 hours), cells were treated with either one of the two footprinting agents (DNaseI and UVC were used), DNA was purified, and ligation-mediated PCR was carried out for the p21, GADD45, MDM2, XPC and XPE genes. **Results:** No footprints in MDM2, XPC and XPE genes were observed. Only a weak in vivo footprint was observed at 1 hour on p21 and GADD45 promoters. **Conclusion:** So far, we haven't been able to provide evidence of p53 binding to its consensus sequence under physiological condition. This could suggest that other factors maybe implicated in vivo that are present in ML-1 cell line and absent in normal human fibroblasts.

IntroductionObjectiveMethodsResultsConclusion*In vivo in vitro.*

Searching for candidate genes influencing genetic susceptibility to asbestos-related diseases. *M.A. Pershouse, A. Groves, C. Schwanke, E.A. Putnam.* Biomed & Pharmaceutical Sci, Univ Montana, Missoula, MT.

The exposure of Libby MT residents to asbestos-contaminated vermiculite is well known. The effects of genetic susceptibility on the development of asbestos-related diseases (ARD) are of primary interest. A mouse model of asbestos exposure is being used to determine gene expression responses to various asbestos forms using a small set of known toxic response genes. It is our hypothesis that asbestos-responsive genes will provide a better understanding of response mechanisms and identify candidate genes for polymorphism analysis of DNA from residents exposed to asbestos. C57Bl/6 mice were exposed via intratracheal instillation of tremolite, winchite, and chrysotile asbestos, in addition to saline and wollastonite, a control fiber. Libby asbestos is an amphibole fiber most closely related to winchite. Other fibers were used as closely related controls. 3 months following exposure, mice were sacrificed and whole lungs harvested for microarray analysis. Using an arbitrary cutoff of 1.5 fold change we found 170 out of 1200 genes affected among the three types of asbestos tested. After tremolite exposure, expression of 41 genes escaped the 1.5 fold cutoff. Winchite exposure resulted in 4 genes being filtered to this level. Exposure to chrysotile (the most common asbestos used in industry) resulted in increased expression of 31 genes, and decreased expression of 5 genes. Therefore, using microarray techniques, a list was generated of genes whose RNA expression levels were specifically altered in response to different asbestos exposures. Among the genes altered in winchite exposure are catechol-o-methyltransferase (Comt), Regulator of G protein signaling 7 (RGS7), macrophage receptor with collagenous structure (Marco), and calmodulin 3 (Calm3). Candidate gene expression changes are being validated at the protein level using immunohistochemistry of corresponding tissue sections. These experiments have provided new candidates for genes involved in the asbestos response pathways. These results will be validated by the analysis of polymorphisms in these identified genes in patient samples. Supported by CCR822092.

Automated mRNA splice site mutation analysis by information theory. *V. Nalla¹, P.K. Rogan^{1,2}*. 1) Schools of Computer Science and Engineering and; 2) Medicine, Children's Mercy Hospital, University of Missouri-Kansas City.

We present a software tool to assist in interpretation of non-coding sequence variation in functional elements within human genes. This secure web server analyzes changes in information content at binding sites due to one or more mutations in any catalogued human gene, genome-mapped mRNA or user-defined sequence [<https://splice.cmh.edu>]. Individual information analyses with splice junction binding site matrices detects activated cryptic splice sites, associated splicing regulatory sites and distinguish null alleles from those that are partially functional. Standard gene and mutation nomenclature (HUGO-approved format) are entered using a CGI-based front end supported by backend server. Back-end software verifies the input variant or haplotypes and retrieves the corresponding hnRNA sequences from the reference genome sequence. The software then substitutes either a mutation(s) for the reference sequence and computes the resulting information contents of all preexisting and altered splice sites and/or regulatory sequences. Changes in information content are tabulated and visualized as walker figures showing the binding sites with the sequence. This application has been validated by analysis of ~400 consecutive mutations published in the journal, *Human Mutation*. We confirmed that all of the previous recognized splicing mutations affected splice site strength or activated cryptic splice sites. At least one missense mutation was also classified as a cryptic splicing mutation since it simultaneously abolished a natural splice donor while activating a down stream pre-existing cryptic site. The long term goal is the development of a turnkey system to predict disease severity of splicing mutations.

Isolation and analysis of primate telomeric regions. *Y. Kuroki¹, A. Toyoda¹, T. Taylor¹, T. Itoh², M. Hattori^{1, 3}, Y. Sakaki¹, A. Fujiyama^{1, 4}.* 1) RIKEN Genomic Sciences Center, Yokohama, Kanagawa, Japan; 2) Mitsubishi Research Institute Inc., Chiyoda-ku, Tokyo, Japan; 3) Kitasato University, Sagami-hara, Kanagawa, Japan; 4) National Institute of Informatics, Chiyoda-ku, Tokyo, Japan.

Sequencing of the human genome is considered finished, however some complex regions such as the centromeres and telomeres still remain. Genomic analysis of these complicated structures is important for understanding the biological and biomedical functions of these regions. To elucidate the biological and evolutionary aspects of genomic structure in primate telomeric regions, we have developed a screening system for isolation of telomeric clones. We have constructed resources, such as sheared fosmid/cosmid libraries constructed from flow-sorted human or primate chromosomes or whole-genomic DNA, and technologies to specifically isolate clones containing telomeric motifs at either end of the DNA insert. This methodology has proven quite effective through successful isolation of telomeric clones from human chromosomes, 11, 18, and 21, all of which our group has helped to complete the sequencing of. Unlike the half-YAC cloning technology, which was developed to clone chromosomal ends using yeast cells that have a high host recombination rate, our technology utilizes an F-plasmid based vector which is similar to the BAC cloning system, thereby ensuring stability and a uniform recombination frequency in the host *E. coli* cell. Both ends of the inserted DNA fragment for each isolated clone were sequenced and mapped onto the human genome based on sequence similarity, or through FISH analysis using human or primate chromosomes. In addition, some clones have been completely sequenced and opened to the public. We plan to continue isolating, mapping, and sequencing additional telomeric clones to better understand the aspects of the telomeric and subtelomeric regions. We are also conducting comparative analysis using telomeric clones isolated from other primates and other organisms in order to track the evolutionary paths of each chromosome. This data will help to complete the tiling path for each chromosome, thus improving tools for diagnosis of chromosomal abnormalities.

Genetic investigation of the acute adverse reactions associated with Angiotensin I-Converting Enzyme Inhibitors.

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Angioedema (AE) and anaphylactoid reactions (AR) are acute adverse effects associated with angiotensin I-converting enzyme inhibitors (ACEi), drugs used for the treatment of cardiovascular diseases. Previous reports have documented significantly lower plasma aminopeptidase P (APP) activities in patients with a history of ACEi-induced AR or AE, which correlate with significantly reduced degradation of des-arginine⁹-bradykinin. Given that the incidence of these adverse reactions differs among ethnic groups, we propose that this metabolic anomaly is partially regulated by genetic factors. To determine the genetic basis of variable plasma APP activity, we estimated the heritability of this quantitative trait using the SOLAR (Sequential Oligogenic Linkage Analysis Routines) program. This predicted that 45.6% of the phenotypic variation results from genotypic differences among members of 8 Caucasian families (total of 118 individuals). The 5 largest pedigrees were subsequently used in a genome scan that consisted of approximately 400 microsatellite markers spaced at genetic intervals of 10 centimorgans. Linkage analysis of the genotypes using SOLAR provided LOD scores greater than 1.4 for 5 loci localized on chromosomes 6, 8, 10 and X. Genotyping of these markers in additional families is underway to determine if these represent true or spurious linkage. In addition, screening of candidate genes is ongoing to identify mutations responsible for reduced plasma APP activity and predict susceptibility to ACEi-induced adverse effects.

GENE EXPRESSION PROFILING OF PATHOGENESIS SEEN IN A MOUSE MODEL OF OBLITERATIVE BRONCHIOLITIS. *J. Lande, S. Dalheimer, M. Hertz, R. King.* Department of Medicine and Institute of Human Genetics, University of Minnesota, Minneapolis, MN 55455, United States.

Background: Lung and heart-lung transplantation are effective treatments for many diseases unresponsive to other therapy; however, long term survival of recipients is limited by the development of obliterative bronchiolitis (OB). We have used a heterotopic murine tracheal transplant model of OB to study the immunological and fibroproliferative events associated with this process, using gene expression microarray analyses to profile the different stages of its development. Methods: Four tracheas from BALB/c (isograft) or C57B6 (allograft) mice were heterotopically transplanted into recipient BALB/c mice. Transplanted tracheas were extracted from recipients at day 4 (epithelial loss/regrowth), 14 (immune response) or 25 (fibroproliferation) for whole graft RNA. Five replicates of each graft type/day combination were collected and RNA expression was analyzed using Affymetrix murine 430A arrays. Two-way ANOVA using graft type and day as factors was performed and transcripts with strong statistical significance were further analyzed. Results: 174 transcripts with F test p-values < 0.00001 were visualized using two dimensional hierarchical clustering. Samples clustered into four prominent groups representing both graft types at 4 days, 14 and 25 day isografts, 14 day allografts and 25 day allografts. Cluster analysis indicated a group of 6 genes associated with cell-mediated immunity with relatively high expression in 14 day allografts, 4 genes with relatively low expression in 14 day allografts and 14 genes associated with humoral immunity that were highly expressed in 25 day allografts. The remaining 150 genes indicated a pattern of moderate expression in 4 day grafts, low expression in later allografts and high expression in later isografts. Conclusions: There are clear and reproducible gene expression differences that differentiate isografts and allografts at 14 and 25 days. These expression profiles appear to reflect known processes associated with OB and offer insight into other less well understood aspects of this disease process.

Recent evolution of the most intact retrovirus in the human genome. *J. Lenz¹, D. Heslin¹, A. Ene¹, G. Turner¹, M. Barbulescu¹, M. Su¹, M. Jensen-Seaman², K. Kidd².* 1) Dept. of Molecular Genetics, Albert Einstein College of Med, Bronx, NY; 2) Dept of Genetics, Yale University School of Medicine, New Haven, CT.

Of the many retroviruses that comprise about 8% of the human genome, HERV-K is the most recently acquired in human evolution and is by far the most intact. Since first entering the catarrhine lineage after the catarrhine-platyrrhine divergence, HERV-K appears to have reinfected the human genome continuously until recently.

We asked when the HERV-K proviruses that are in the human genome today originally formed. HERV-K proviruses exist in three forms, relatively intact, full-length viral genomes of which there are about 20 in the human genome today, partial viral genomes, and solo LTRs, a product of intraviral homologous recombination. Using a method to date the time of formation solo LTRs (that far outnumber the full-length proviruses), we estimate that about 100 HERV-K proviruses were fixed in the human genome since the human and chimpanzee lineages diverged. Most of these formed within the most recent 1.2 to 2.7 million years, suggesting that HERV-K has been relatively active during this period of time.

One HERV-K provirus is present at the orthologous positions in the gorilla and chimpanzee genomes, whereas humans contain an intact preintegration site. This provides an excellent example of a locus where the gorilla and chimpanzee genomes are more like each other than like that of humans.

At least 13 of the full-length HERV-K proviruses in the human genome formed after the human and chimpanzee lineages diverged. At least three formed sufficiently recently that they exist allelically with the intact preintegration sites, and each is present in only a fraction of humans today. Many solo LTRs also exist allelically with the cognate preintegration site. One of the proviruses contains full-length open reading frames for all viral proteins. This is the best candidate to be a human genome retrovirus that is capable of reinfected humans today.

The palindromic content of the human genome. *S. Lewis*^{1,2}, *T. Allyn*³, *J. Cheung*¹. 1) Hospital for Sick Children Toronto, ON, Canada; 2) University of Toronto, Toronto, ON, Canada; 3) University of Waterloo, Waterloo, ON, Canada.

True palindromes (comprised of a sequence that is immediately followed by its inverse complement) are hotspots of DNA rearrangement. Under the stress of hypernegative supercoiling, a DNA palindrome can assume an intra-strand paired cruciform structure. Such branched DNA is functionally compromised; cruciformation not only interferes with DNA replication and transcription but also generates a substrate for nucleases and recombinases. Palindromy confers a latent propensity for damage on a DNA sequence, damage that is manifested under some circumstances, though not others.

The study of DNA palindromes longer than about 200bp is impeded by a profound technical barrier which is that such sequences are impossible to clone in *E. coli*. Thus any long, true palindromes that might occur naturally in the human genome are systematically excluded from the sequenced record. We are interested to know whether long, true DNA palindromes exist in human DNA and if so, whether such sequences are associated with pathological DNA rearrangement.

To determine whether long true palindromes exist, we undertook a two-part study. First, we looked for evidence of palindromy by searching the human genome for all instances of palindromes longer than 90 bp. We were able to find 67 putatively palindromic sites, few of which exceeded 100bp. Next we took representative palindromic hits from this computationally-defined collection and tested for their presence or absence among normal individuals. This was achieved by PCR, cloning and DNA sequence analyses.

In brief, inter-individual comparisons show a high level of polymorphism for at least some palindrome-bearing loci. Moreover, different polymorphic forms of the same locus have profoundly different biophysical properties. These studies verify that sites of putative palindromy are not assembly or sequencing errors, but do indeed exist in the human genome. Moreover, the presence of additional "hidden" palindromes is strongly implied by the length cut-off we observed.

Evidence that simple sequence repeats influence the replication time of genes. *M. Regelson*¹, *D. Eller*¹, *Y. Marahrens*^{1, 3}, *S. Horvath*^{1, 2, 3}. 1) UCLA Department of Human Genetics; 2) UCLA Department of Biostatistics, Gonda Center, David Geffen School of Medicine, 695 E. Young Drive South, Los Angeles, CA 90095-7088, USA; 3) Co-directed this work.

Chromatin context determines when a gene replicates in S phase. Genes display a variety of characteristic replication times and transcription factors must cope with the chromatin milieu associated with each gene. Little is known about what is responsible for this diversity. Here we present evidence that SATellite-Related sequences (SARs) and tracts of simple sequence repeats (SSRs), in particular (CA)_n and (ACTG)_n, are increasingly abundant in the regions flanking later replicating genes in *Drosophila* while (CATA)_n tracts are increasingly abundant around earlier replicating genes. Each (ACTG)_n tract, (CATA)_n tract, and SAR influences replication time over a ~100-kb region while each (CA)_n tract influences replication time over ~50-kb. Although these four repeats comprise less than one half of one percent of the "euchromatic genome" in *Drosophila*, they account for ~7% of the variation of gene replication timing and (CA)_n alone appears to influence more than 80% of genes. We propose that SSRs exist to impart heterochromatic properties on basal or default chromatin in order to suppress unwanted transcription and that genes have evolved mechanisms to open up localized regions within this chromatin for transcriptional activation.

The search for genetic modifications of systemic lupus erythematosus through expression profiling of peripheral blood mononuclear cells. *F. Andrade*¹, *S.D. Ghimbovschi*², *D. Alarcon-Segovia*¹, *E.P. Hoffman*². 1) Instituto Nacional de Ciencias Mdicas y Nutricin Salvador Zubiran, Mxico, DF;; 2) Center for Genetic Medicine, Children's Research Institute, Washington, DC.

Systemic lupus erythematosus (SLE) is a complex and heterogeneous disease of unknown etiology, characterized for high titer antibody response against self-antigens. Recent studies have addressed the molecular signatures that characterize lupus during flaring (e.g. the interferon and granulopoiesis signatures). However, it has not been defined whether patients with SLE have a primary defect responsible of these signatures. To define primary immunological abnormalities in SLE, we propose that any functional problem (either genetic or epigenetic) associated with lupus must be detectable independently of whether the disease is active or inactive (i.e. remission). However, the advantage of studying SLE patients in remission is that it can allow dissecting different pathways involved in SLE pathogenesis, in the absence of the inflammatory background caused during flaring. Therefore, to better understand functional mechanisms associated with SLE, we compared the molecular response of PBMCs from SLE patients in remission and healthy individuals, which were exposed to a similar "lupus environment" at different time points: 0, 12, 24 and 48 h. SLE patients were in treatment-free remission for at least a year, having had a severe form of disease in the past. As lupus environment, we used plasma from 2 patients with active SLE not yet treated. In addition, PBMCs were incubated with autologous plasma to define background gene expression. Expression changes were evaluated for the 26,244 genes and ESTs represented on the Affymetrix HG_U133 plus 2.0 GeneChip. Our data suggests that the heterologous plasma from SLE patients in the active stage of the disease have protein factors that induce an activation of the immune system via a cascade of immune response genes (MHC class II-DQ, proapoptotic genes, Ig-lambda, complement C2, interferon-induced proteins, etc.) that yield both common and different activation signature patterns in healthy controls and in SLE patients in remission.

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Examining a Comprehensive System for Fragment-based Applications. *L. Joe, S. Karudapuram, C. Kosman, Y. Lou, T. McElroy, A. Wheaton.* Applied Biosystems, Foster City, CA.

The completion of the Human Genome Project has introduced a new paradigm in complex disease research. With an increasing emphasis on the need to rapidly scan the genome for variations, researchers need tools that will enable discovery and detection of genetic variation in an efficient and highly automated fashion. Since genetic variation can be characterized using a variety of methods it is important that detection and analysis systems are flexible and can easily transition from one application type to the next.

In this study, we describe recent innovations in Applied Biosystems system approach to fragment analysis applications. We demonstrate improved data quality with new matrix and size standards, a highly optimized suite of fragment-specific run modules, and single polymer-array combinations for multiple applications. We also illustrate several software features that improve the overall system flexibility and efficiency, including improved data collection run scheduling and prioritization, multi-application plate support, and increased functionality in analysis software for support of a broader range of fragment-based applications.

Computational prediction of chromatin structure in vivo. *W.S. Noble¹, R. Humbert², J. Wallace², M. Yu³, M. Hawrylycz², J. Stamatoyannopoulos².* 1) Genome Sciences, University of Washington, Seattle, WA; 2) Dept of Molecular Biology, Regulome, 551 N 34th St, Seattle, WA 98103; 3) Division of Medical Genetics, Univ. of Washington, Seattle, WA 98195.

Computational prediction of cis-regulatory sequences in the context of complex genomes has proven to be a major challenge. Focal chromatin remodeling is a general property of human cis-regulatory sequences, which are marked by DNaseI hypersensitive sites (HSs) in vivo[1]. The ability to discriminate HSs from genomic sequences that reside in unremodeled chromatin using a computational algorithm would dramatically facilitate the identification of functional non-coding elements in the human genome. We trained a support vector machine (SVM) classifier[2] using a set of DNaseI HS sequences (n=280) and non-HS control sequences (n=737), and tested the ability of the SVM to predict HSs ab initio in the human genome. We found that the SVM was able to discriminate in vivo HSs with high accuracy (>70%) in the context of both erythroid cells. 89% of the HSs detected in erythroid cells were also found in lymphoid cells, indicating that the SVM was detecting a class of sequence elements that are widely involved in control of chromatin structure, and, by extension, gene regulation. The results provide the first evidence for the feasibility of large-scale computational prediction of chromatin structure in vivo and suggest the existence of common sequence features that dictate chromatin accessibility to the regulatory machinery across the human genome.

Outcome of orthotopic liver transplantation (OLT) in children with inborn errors of metabolism (IEM). *G.L. Arnold, J. DeLuca, R. Bowman.* Div Pediatric Genetics, Univ Rochester Sch Medicine, Rochester, NY.

We reviewed the course of all five patients undergoing OLT for IEM at the University of Rochester School of Medicine and Dentistry. Patient ages/diagnoses included urea cycle disorders (Ornithine Transcarbamylase Deficiency (OTC), male age 18 mos and female age 3 yrs; Argininosuccinate Lyase Deficiency (ASL) age 20 mos), Tyrosinemia Type 1 (Tyr 1) age 6 yrs and Methylmalonic Acidemia (MMA) age 7 yrs. Transplant indications included recurrent episodes of hyperammonemia (urea cycle disorders), suspicious liver nodules (Tyr 1) and recurrent pancreatitis (MMA). The 3 y.o. with OTC received a lobe of a parent's liver; others received cadaver organs. The mean (median) number of inpatient days was: for the year before transplantation 78 (16) days (range 0-197), for the transplant admission 38 (13) days (range 9-86), and for the post transplant year (excluding the transplant admission) 22 (28) days (range 0-41). A complex pre-transplant course tended to predict more post-transplant complications. For the urea cycle patients preoperative intravenous ammonia diverting drugs were stopped on removal of the native liver. Ammonia at the start of surgery ranged from 25-196 $\mu\text{mol/l}$ and was below 40 post-operatively; no further treatment for hyperammonemia was required. The MMA patient was hemodialyzed for 4 hours prior to transplant to reduce abnormal metabolite levels; post transplant both pancreatitis and renal function improved (although mild renal insufficiency persists) as did the lifelong profound anorexia. In the year after transplant, growth percentiles were generally predicted by severity of medical complications. Of interest some patients demonstrated catch up linear/weight growth, and head circumference percentiles increased in the two youngest patients. Developmental delay improved but did not normalize in the two significantly delayed children (ASL and MMA). Overall, parents believed their child's quality of life was improved compared to before the transplant and none regretted the procedure. Risks of OLT must be considered, but the procedure can provide an improved medical and psychosocial quality of life for children having significant IEM.

The C677T mutation in the MTHFR gene follows a clinal distribution within Pakistan. *Q. Ayub, A. Mansoor, S. Rehman, S.Q. Mehdi.* Biomedical and Genetic Engineering Laboratories, Islamabad, Pakistan.

The enzyme, 5,10-methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of 5, 10 methylene tetrahydrofolate to 5-methyltetrahydrofolate, the carbon donor for the re-methylation of homocysteine to methionine. The C to T transition at position 677 (C677T) of the MTHFR gene replaces the amino acid alanine with valine at position 222 making the enzyme thermolabile and reducing its activity in the heterozygous or homozygous state, thus causing hyperhomocysteinaemia. The frequency of this mutation in the Pakistani population, that suffers from several nutritional and vitamin deficiencies, is unknown. In this study we examined the frequency of this mutation in 542 Pakistani individuals belonging to 14 ethnic groups. The C to T substitution creates a *HinfI* restriction site and was detected by the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism method. The frequency of the T allele was significantly higher in populations from northern Pakistan (t test statistic =8.585; p=0.019). The highest frequencies were observed in the Hazara (0.34), Kalash (0.27) and Meo (0.28) ethnic groups. Among the southern populations only the Parsi, who are migrants from northern Iran, have a high frequency of the T allele (0.21). The higher frequency of the mutant allele was significantly correlated with longitude (r=0.582; p=0.037) and latitude (r=0.686; p=0.010). The cline appears to be between populations that live in the plains and have plentiful supply of food throughout the year as opposed to those populations that live in the arid mountainous or desert regions of Pakistan. However, the higher frequency of the T allele could also be due genetic drift in isolated populations such as the Hazara, Kalash and Meo. The Hazara have undergone several population bottlenecks in their history and the Kalash have remained isolated in the Hindukush Mountains for centuries. The possibility that these populations could be susceptible to developing hyperhomocysteinaemia and its cardiovascular consequences are major health concerns.

Phenylalanine hydroxylation in patients with classic PKU in response to tetrahydrobiopterin. *O.A. Bodamer, M. Holub, B. Hagerty, A. Muhl, K. Tuschl.* Department of Biochemical Genetics, University Children's Hospital Vienna, Austria.

INTRODUCTION: A significant number of patients with HPA and mild PKU and a limited number of patients with classic PKU have been shown to be responsive to tetrahydrobiopterin (BH4) resulting in decreased plasma phenylalanine levels and increased dietary phenylalanine tolerance. The observed effects were attributed to stimulation of phenylalanine hydroxylase (PAH) by its cofactor BH4.

METHODS AND SUBJECTS: A stable isotope method employing ring D5 phenylalanine, ring D2 and D4 tyrosine was used to measure phenylalanine and tyrosine flux and to calculate whole body phenylalanine hydroxylation (PAHtion) rates before and fifteen hours after an oral BH4 load (20 mg/kg) in three adult subjects with classic PKU and three healthy adults. Dietary phenylalanine intake was kept unchanged.

RESULTS: Plasma phenylalanine levels decreased in all PKU patients: 532, 990 and 1120 mol/l before versus 426, 819 and 924 mol/l after BH4 load while there was no change in the control group. Phenylalanine hydroxylation rates remained unchanged in both groups: 14.2, 9.9, 13.0 versus 15.0, 10.9 and 11.4 mol/kg/h in subjects with PKU and 13.7, 13.5, 8.3 versus 13.8, 14.6, 15.8 mol/kg/h in controls, before and after BH4 load.

DISCUSSION: Although there is no change in PAHtion following a BH4 load plasma phenylalanine levels decreased substantially in subjects with classic PKU. This may be due to increased protein synthesis and/or phenylalanine break down by yet unknown mechanisms. Interestingly absolute PAHtion was similar between PKU and controls but when corrected for plasma phenylalanine levels PAHtion was 5-10 fold lower in PKU. Some of the apparent residual activity of PAH may be in fact due to tyrosine hydroxylation. Although the number of patients and controls studied is limited the results demonstrate that it is feasible to employ a stable isotope method to measure PAH activities in response to a BH4 load.

Functional characterization of a third mitochondrial ornithine transporter gene in the mouse. *J.A. Camacho, N. Rioseco-Camacho, D. Andrade.* Pediatrics, University of California, Irvine, CA.

Redundant genes that can rescue a disease phenotype are potential modifier genes that can influence disease onset, severity and in part be responsible for phenotypic variability. We previously demonstrated that human ORNT2 (HsORNT2), a functional retroposon with 88% identity to human ORNT1 (HsORNT1), can rescue the deficient ornithine transport in cultured fibroblasts of patients with the Hyperornithinemia-Hyperammonemia-Homocitrullinuria (HHH) Syndrome, a disorder of the urea cycle and ornithine degradation pathway. Both ORNT1 and ORNT2 are members of the mitochondrial carrier family (MCF) of proteins and belong to a sub-family of cationic amino acid transporters. We initially screened the mouse database of Expressed Sequence Tags (dbEST) for additional MCF genes that phylogenetically belong to the ORNT sub-family utilizing a conserved amino acid sequence (P29FDT) of mitochondrial ornithine transporters from several species. We identified a mouse EST from Chr12 that has a complete open reading frame (ORF) that is 28% identical to the murine ORNT1 gene. The human ortholog is localized in the q32.2 region of chromosome 14. We tentatively named this gene PFDT2. Both the human and mouse genes have three introns interrupting their ORFs. Northern blotting, PCR and dbEST analysis demonstrated that PFDT2 is predominantly expressed in the CNS with lesser expression in the liver, lung, kidney and heart. This is in contrast to the HsORNT1 and HsORNT2 genes that are predominately expressed in the liver and pancreas. Overexpression (n=3 experiments) of an N-myc-MmPFDT2 construct in cultured HHH fibroblasts rescued the deficient ornithine metabolism of these cells. Immunofluorescence experiments confirmed that MmPFDT2 targets solely to the mitochondria. Our results suggest that the PFDT2 gene codes for a mitochondrial cationic amino transporter that is most likely involved in CNS function and possibly the ornithine degradation pathway.

Identification of new mutations within TATc gene in tyrosinemia type II Tunisian patients. *C. Charfeddine¹, K. Monastiri², M. Mokni³, A. Laadjimi⁴, M. Kairallah⁴, N. Kaabachi⁵, N. Tebib⁵, MF. Ben Dridi⁶, K. Dellagi¹, S. Abdelhak¹.* 1) Immunology, Institut Pasteur, Tunis, Tunisia; 2) Departement of Pediatrics, Hospital of Monastir, Monastir, Tunisia; 3) Departement of Dermatology, Hospital of La Rabta, Tunis, Tunisia; 4) Departement of Ophthalmology, Hospital of Monastir, Monastir, Tunisia; 5) Laboratory of Biochemistry, Hospital of La Rabta, Tunis, Tunisia; 6) Departement of Pediatrics, Hospital of La Rabta, Tunis, Tunisia.

Tyrosinemia type II or Richner Hanhart Syndrome (RHS) is an autosomal recessive disorder characterized by keratitis, palmoplantar keratosis, mental retardation, and elevated blood tyrosine levels. The disease is due to a deficiency of hepatic cytosolic tyrosine aminotransferase (TATc), an enzyme involved in the tyrosine catabolic pathway. The majority of reported cases are found among the Arab and Mediterranean populations probably because of the high rate of consanguineous mating. RHS is characterized by inter and intrafamilial phenotypic variability. A large spectrum of mutations within TATc gene has been shown to underlie RHS. Our aim is the molecular and genetic investigations of RHS in 3 unrelated consanguineous Tunisian families with 7 patients with confirmed biochemical diagnosis for tyrosinemia type II. Families were genotyped with five polymorphic microsatellite markers overlapping the TATc gene. The haplotype analysis revealed that all RHS patients were homozygous for the 5 tested markers. Two haplotypes were observed, suggesting that at least 2 different mutations within TATc gene segregate with these 2 haplotypes. Mutations analyses were performed and two novel missense mutations were identified (C151Y) and (L273P) within exon 5 and exon 8 respectively. Phenotype variability was observed even among individuals sharing the same mutations.

Separation and quantification of branched chain amino acids by LC/MS/MS. *C.L. Garganta, D.J. Czyzyk.* Dept of Genetics, Yale School of Medicine, New Haven, CT.

Maple syrup urine disease (MSUD) is due to deficiency of branched chain ketoacid dehydrogenase. It results in the accumulation of branched chain amino acids and allo-isoleucine, a stereoisomer of isoleucine. Dietary restriction of branched chain amino acids is the mainstay of MSUD therapy and requires frequent monitoring of branched chain amino acid concentrations. To allow collection of blood samples at home, we developed a method to measure branched chain amino acids and allo-isoleucine in blood collected on filter paper.

The amino acids are quantified in a 1/8 inch blood spot by liquid chromatography/tandem mass spectrometry (LC/MS/MS). Samples are extracted with an internal standard solution containing d₈-valine and d₃-leucine in methanol.

The underivatized amino acids are separated on a short C8 column using 25% methanol and 75% 0.1 mM pentadecafluorooctanoic acid. 20% of the column effluent is introduced via the TurboIonSpray into an API2000 triple quadrupole MS/MS. The following transitions were monitored in positive ion MRM mode: 118 to 72 (valine), 126 to 80 (d₈-valine), 132 to 86 (leucines), and 135 to 44 (d₃-leucine). Total run time is 3.5 minutes.

Recoveries were 95-105% across a broad range of concentrations. Interassay coefficient of variation was 5-10%. More than 60 simultaneously collected plasma and blood spot samples were analyzed by amino acid analyzer and this method, respectively. The concentration determined by LC/MS/MS was plotted against concentration measured by amino acid analyzer. The slopes for all 4 compounds were between 0.9 and 1.1 with correlation coefficients >0.95.

This method provides accurate measurement of branched chain amino acid concentrations in blood spot samples. It allows home collection of blood samples for monitoring of MSUD and disorders of leucine metabolism. This testing could also be used in newborn screening labs as a second tier test to eliminate false positive results since the absence of allo-isoleucine completely excludes a possible diagnosis of MSUD.

Neonatal mass screening for hereditary tyrosinemia type I by succinylacetone measurement: The Quebec experience. *Y. Giguere, J. Ruel, N. Belanger, A. Grenier, C. Laberge.* Quebec Neonatal Blood Screening Programme, CHUL du CHUQ, Quebec, Canada.

Hereditary tyrosinemia type I (HTI) is a rare autosomal recessive disorder caused by a fumarylacetoacetate hydrolase (FAAH) deficiency characterized by hepatic failure, renal dysfunction and neurological crisis. The prevalence of HTI is increased in the province of Quebec (1/15000-20000) due to a founder effect in the French Canadian population. Thus, HTI testing is included in the neonatal screening program. During the 1970-1997 period, 2249000 newborns were tested at 3-7 days of life on dried filter paper blood spots for tyrosine (Tyr) level followed by semi quantitative and quantitative (Q) indirect succinylacetone (SA) measurement (-aminolevulinate dehydratase inhibition), and immunoreactive FAAH as the confirmatory test. During that period, 118 cases were detected (HTI incidence = 1/19000), but owing to earlier sampling and increased incidence of breastfeeding, the Tyr cut-off was lowered gradually from 414 to 200 mol/L (7.5 to 3.6 mg/dl), decreasing the discriminating power of blood Tyr. Moreover, since 1996, due to increasing pressure to discharge the mothers and their newborns within 2-3 days after delivery, early Tyr measurement was not sensitive enough as proven in the 5 cases missed (with Tyr 200 mol/L). Since 1998 (1998-2004; 91,7% of sampling performed before 72 hrs of life), we thus modified the testing sequence: 436260 newborns have been tested by semi-Q SA measurement (normal 0.20 O.D.) followed by Q-SA testing (normal 2.5 mol/L) and FAAH as the confirmatory test, Tyr being an auxiliary test. During that period, only 59 (0.02%) neonates showed abnormal results, 36/59 normalized in the repeat sample (0,01% false positive rate), while 23/59 were confirmed by abnormal Q-SA and FAAH, and clinically to have HTI (incidence: 1/15773). No false negative results were reported. Tyr level of cases (n = 23) was 197 77 mol/L, below the 200 mol/L threshold, and 151 51 mol/L in normal newborns (n = 436,201). Thus, since 1998, SA measurement as the initial HTI screening test proved to be highly sensitive and specific despite earlier sampling of neonates.

Wild-type phenylalanine hydroxylase activity is enhanced by tetrahydrobiopterin supplementation *in vivo*: an implication for therapeutic basis of tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency. S.

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Phenylketonuria (PKU) is caused by phenylalanine hydroxylase (PAH) deficiency. A phenylalanine (Phe)-restricted diet can ameliorate the effects of high serum Phe on cognitive function. However, Phe restriction is a heavy burden to patients and their families and better therapeutic approaches have been explored. We previously proposed a novel disease entity, tetrahydrobiopterin (BH4)-responsive PAH deficiency, in which administration of BH4 reduced elevated levels of serum phenylalanine (Kure S et al., *J Pediatr*, 1999;135:375-8). Subsequent reports indicate that the prevalence of BH4-responsive PAH deficiency is much higher than initially anticipated. Although growing attention surrounds treatment with BH4, little is known about the mechanism of BH4 responsiveness. An early report indicates that BH4 concentration in rat liver was 5 M where *Km* for BH4 of rat PAH was estimated to be 25 M in an oxidation experiment using a liver slice, suggesting relative insufficiency of BH4 in liver. In the present study, we developed a breath test using [1-¹³C]phenylalanine in order to examine the BH4 responsiveness of normal mouse PAH *in vivo*. The reliability of the test was verified using BTBR mice and its mutant strain lacking PAH activity, *Pah^{enu2}*. BH4 supplementation significantly enhanced ¹³CO₂ production in C57BL/6 mice. Furthermore, BH4 apparently activated PAH activity in just five minutes. These observations suggest that submaximal PAH activity occurs at the physiological concentrations of BH4 normally present in hepatocytes *in vivo*, and that PAH activity can be rapidly enhanced by supplementation with BH4. Thus, we propose that the responsiveness to BH4 in patients with PAH deficiency is due to the fact that suboptimal physiological concentrations of BH4 are normally present in hepatocytes and the enhancement of the residual activity may be associated with a wide range of mutations.

Leukodystrophy in presumed late-onset cobalamin C disease. *C. Thauvin-Robinet¹, G. Couvreur², S. Giraudier³, D. Perrenou⁴, J. Desgres⁵, T. Moreau², M. Giroud², L. Faivre¹.* 1) Centre de Genetique, Hopital d'Enfants, Dijon, France; 2) Service de Neurologie, Hopital General, Dijon, France; 3) Service d'Hematologie-Biologique, Hopital Henri Mondor, Paris, France; 4) Centre de Convalescence et de Reeducation, Dijon, France; 5) Laboratoire de Biochimie specialisee, Hopital d'Enfants, Dijon, France.

Combined methylmalonic aciduria and homocystinuria cobalamin C type are the most common of the inborn errors of cobalamin metabolism. Clinical features occur usually in the first year of life including failure to thrive, poor feeding, hypotonia, vomiting, anemia, hypersegmented neutrophils, thrombocytopenia, mild to moderate developmental delay, microcephaly, and seizures. Only a few cases with late onset are described in the literature. A 39 year-old woman was referred to our unit for the association of progressive neurological deterioration, mental confusion, myoclonia and visual hallucinations. Familial history was unremarkable. Clinical history found normal psychomotor development in childhood but onset at 15 years of age with behavior problems. Pulmonary embolism with hemiplegia, depression and anorexia occurred after delivery at 19 years of age. Seizures were noted at 31 years of age. Workup for causes of neurological deterioration included cerebral MRI showing diffuse leukodystrophy with cortical atrophy and metabolic investigations showing severe hyperhomocysteinemia and low plasma methionine levels in favor of inborn errors of cobalamin metabolism. Urine increased methylmalonic acid levels ruled out MTHFR deficiency. Impaired synthesis of adenosylcobalamin and methylcobalamin in cultured skin fibroblasts was found. Complementation studies are in process, a cobalamin C disease being the most likely diagnosis. The patient was treated by folic acid, betane and hydroxycobalamin, showing partial improvement of the symptomatology. The two families with late-onset cobalamin C disease reported in the literature are reviewed, with a neurological onset between 12 and 16 years of life. These observations show that aminoacids chromatographies should be part of the investigations of unexplained neurological symptoms in adults.

Founder Effect of Phenylketonuria in the Geauga County Amish Settlement of Ohio. *H. Wang¹, L. Nye¹, E. Puffenberger², H. Morton².* 1) Das Deutsch Center (DDC) Clinic for Special Needs Children, Middlefield, OH; 2) The Clinic for Special Children, Strasburg, PA.

Phenylketonuria (PKU) is a metabolic inborn error resulting from a deficiency of phenylalanine hydroxylase (PAH) (EC 1.14.16.1). Over 400 causative mutations have been identified in the past. It seems to be a lower-frequency founder disorder in the Amish population of Lancaster County, appearing less commonly than in the general population. However, here we report that the higher incidence of PKU recently documented in the Geauga County Amish Settlement of Ohio is associated with a founder effect. In this report, 15 individuals with classic PKU were identified from five families. The incidence was greater than 1/1000 in this community. Genealogic analysis showed that all patients could be traced back seven generations to the common ascendants Jeremiah Miller (1817-1890) and Lydia Troyer (1819-1865). DNA was isolated from peripheral blood of four patients in three families in this large pedigree, and the PAH gene was amplified by PCR and then subjected to direct sequencing. We found that all patients were homozygous for the same mutation, IVS10-11 GA. In fact, this is one of the common mutations reported in PKU patients in Europe. As reported earlier, this mutation activates a cryptic splice site and results in PAH protein conformational changes, leading to a non-functional protein. Although we have previously identified this mutation in Mennonite patients, this is the first time we have found it in the Amish population. We are currently in the process of confirming the mutation in all patients. As most PKU patients are compound heterozygotes rather than homozygotes, the cohort of the homozygous patients in this report is highly valuable for further study of the disease from other aspects, such as exploring the role of other genomic factors and environmental factors in the disease phenotypes.

Cobalamin C disease presenting with hemophagocytic lymphohistiocytosis. *S. Yano¹, S. Wu¹, T. Coates², I. Gonzalez³.* 1) Div Medical Genetics, Childrens Hosp, Los Angeles, Los Angeles, CA; 2) Div Hematology/Oncology, Childrens Hosp, Los Angeles, Los Angeles, CA; 3) Dept Pathology and Laboratory Medicine, Childrens Hosp, Los Angeles, Los Angeles, CA.

Patients with methylmalonic aciduria and homocystinuria have defective metabolism of cobalamin to methylcobalamin and adenosylcobalamin. Three complementation groups (cbl C, cbl D, and cbl F) are known in this category. Each cobalamin disease is rare and has autosomal recessive inheritance. The most common one is cbl C disease which is characterized by seizures (often neonatal onset and intractable), developmental delay, megaloblastic anemia, hemolysis, and failure to thrive, in addition to biochemical abnormalities including metabolic acidosis, methylmalonic aciduria and homocystinuria. Hemophagocytic lymphohistiocytosis (HLH) is characterized by infantile onset (80 % of cases occur less than age 1 year) long lasting fever (> 38.5 C, more than 7 days), failure to thrive, hepatosplenomegaly, and anemia as well as characteristic pathologic findings of histiocytes demonstrating phagocytosis of erythrocytes in bone marrow. The Genetic form (Familial HLH, autosomal recessive) and a secondary sporadic isolated form are known: the latter occurs in association with a systemic infection, malignancy or immunodeficiency disorder. Chemotherapeutic agents including cyclosporine and VP-16 are commonly used to treat HLH. It is extremely rare for patients with inborn errors of metabolism to have HLH as one of the clinical symptoms. Four cases of Lysinuric protein intolerance (LPI) have been reported that they fulfilled the diagnostic criteria for HLH (Duval et al., J Pediatr, 1999 134: 236-239). Besides this report, there have been no reported cases of inborn errors of metabolism to have HLH in their clinical courses. We present a case of an infant diagnosed with hemophagocytic lymphohistiocytosis who was later confirmed as having Cobalamin C disease.

Auditory electrophysiology of the *mdx*^{Cv3} mouse shows abnormal latencies which may provide evidence for one basis of cognitive defects found in Duchenne muscular dystrophy. *D.M. Pillers*^{1,2,3}, *J.B. Kempton*⁴, *N.M. Duncan*¹, *S.J. Dwinell*³, *S.M. Rash*¹, *D.R. Trune*⁴. 1) Pediatrics; 2) Ophthalmology; 3) Molecular & Medical Genetics; 4) Otolaryngology - Head and Neck Surgery, Oregon Health & Sci Univ, Portland, OR.

Introduction: Neurosensory electrophysiology is characterized by two major markers: the amplitude of the response to a stimulus, and the time interval until the response peaks. In auditory physiology, this time interval is referred to as the latency, whereas in visual electrophysiology, it is known as the implicit time. We have shown abnormalities in the implicit time of the electroretinogram in the *mdxCv3* mouse model versus the *mdx* which is normal, and sought to determine whether any parallels could be drawn in the auditory pathway. **Methods:** Auditory brainstem response (ABR) audiometry to pure tones was used to evaluate cochlear function. 14 *mdx*, 4 *mdxCv3*, and 13 age-matched control (C57BL/6J and C57BL/10ScSn) male mice were tested. The ABR thresholds to tone-burst stimuli at 4, 8, 16, and 32 kHz were obtained for each ear and statistically compared (ANOVA) for potential group differences. Latencies were determined for wave IV and plotted against intensity. **Results:** Both *mdx* and *mdxCv3* mice demonstrated normal ABR thresholds when compared with controls. However, the latency response curves showed significant differences between the two mouse models. Although the *mdx* curve overlapped with that of the normal mice, that of the *mdxCv3* showed significant shortening of the wave IV latency ($p < 0.05$). **Discussion:** Abnormalities in latency in auditory brainstem response testing can provide evidence for asynchrony in signal processing. Disorders with such asynchrony can be associated with cognitive defects. A subset of patients with Duchenne muscular dystrophy have cognitive defects ranging from dyslexia to mental retardation. Genotype-phenotype correlations in man have shown that 3' DMD mutations, such as that found in the *mdxCv3* mouse, are predominantly associated with the cognitive defects. We speculate that the abnormal latency in the auditory response may provide evidence of a physiologic basis for these cognitive defects.

From deletion to disease: Identification of a novel mutation in the catalytic subunit 1 of the pyruvate dehydrogenase phosphatase (PDP1) gene and demonstration of protein phosphatase regulation. *J.M. Cameron¹, M.C. Maj¹, V. Levandovskiy¹, N. MacKay¹, R. Baumgartner², B.H. Robinson¹.* 1) Dept Metabolism, Hospital Sick Children, Toronto, ON, Canada; 2) Metabolic Unit, Universitäts-Kinderspital beider Basel (UKBB), Postfach, CH-4005, Basel, Switzerland.

Pyruvate dehydrogenase deficiency (PDH; OMIM 312170) is a clinically heterogeneous disorder caused by mutations in the E1alpha, E3 and protein X components. PDH activity is regulated by phosphorylation: it is inactivated by PDH kinase and activated by PDH phosphatase, PDP. Patients have been reported previously that have a reduction in PDH phosphatase activity, but no mutation has ever been identified.

We have identified two brothers with hypotonia, feeding difficulties and delayed psychomotor development with elevated lactate levels. Both patients demonstrated deficient native PDH activity in skin fibroblasts and lymphoblasts. However, activation of the PDH complex using dichloroacetate showed almost normal PDH activity, suggesting there was a possible problem with the efficient regulation of the enzyme complex.

PDP is a dimeric enzyme that consists of a catalytic and regulatory subunit; the catalytic subunit has two isoforms which show differing dependencies to Ca^{2+} ; and Mg^{2+} ;. After sequencing the cDNA of all three genes, we identified a three base pair deletion in the PDP1 gene in both brothers, which was confirmed in genomic DNA.

We have purified human PDP1 and PDP2 proteins and shown them to be active in vitro, and have demonstrated restoration of patient PDH activities after introduction of wild-type PDP1 to fibroblast cell lysates. We are also confirming these findings by gene transfection into intact cells.

Gene expression analysis in the acid sphingomyelinase deficient mouse: Insights into the pathogenesis of Niemann-Pick disease and the identification of new biomarkers. *R. Dhami, E. Schuchman.* Dept Human Genetics, Mount Sinai Sch Medicine, New York, NY.

Acid sphingomyelinase (ASM) deficiency leads to neuronopathic (Type A) and non-neuronopathic (Type B) forms of Niemann-Pick disease (NPD). ASM knockout (ASMKO) mice display neurological and visceral organ defects similar to those documented in human patients, and have been used to investigate various therapeutic strategies. Assessment of therapeutic efficacy, in both humans and mice, is challenged by a lack of knowledge about the mechanisms involved in the development of different tissue pathologies, and by a lack of markers to predict disease severity and progression. To address these issues, we have used oligonucleotide microarray analysis to study differential gene expression in the lungs and brains of ASMKO mice, two important sites of pathology in NPD. The results confirmed the fact that inflammation is an important component of NPD pathogenesis, and also revealed differential expression of several unexpected genes that may provide important clues to the primary pathways involved in NPD tissue injury. For example, in ASMKO mouse lungs the genes encoding several matrix-degrading proteases, including cathepsin K and MMP-12, were elevated. In the brain, expression of the growth hormone gene was markedly increased, while the expression of several other neural-specific genes were decreased. To demonstrate the usefulness of this gene expression data for the identification of possible biomarkers, the serum concentrations of growth hormone, chitotriosidase, serum amyloid A3, and orosomucoid were determined in the ASMKO mice. Both growth hormone and chitotriosidase appear to be good biomarkers of disease progression. Thus, these gene expression analyses have provided valuable information about possible pathogenetic mechanisms in NPD, and identified potential biomarkers that could be used to assess disease progression and therapeutic efficacy.

Canine Mucopolysaccharidosis IIIB: Aspects of Visceral and Brain Pathology. *N. Ellinwood*^{1,4,5}, *L. Verot*², *M.-A. Colle*³, *U. Giger*⁴, *M.T. Vanier*², *M.E. Haskins*⁴. 1) Labo de Thérapie Génique, CHU Hôtel-Dieu, Nantes, France; 2) INSERM U 189 and Fondation Gillet-Mérieux, Lyon-Sud Medical School and Hospital, Pierre-Bénite, France; 3) Labo d'Anatomie-Pathologique, Ecole Nationale Vétérinaire de Nantes, France; 4) Depart's of Clinical Studies and Pathobiology, Univ of Penn, Philadelphia, PA; 5) Depart of Animal Sci, Iowa St Univ, Ames, IA.

Mucopolysaccharidosis (MPS) III comprises a group of severe neuropathic lysosomal storage diseases characterized by a failure of heparan sulfate catabolism. The cause of MPS type IIIB is deficient activity of N-acetyl--D-glucosaminidase (Naglu). Clinically, MPS IIIB is characterized in humans by mild somatic features and severe central nervous system (CNS) deterioration with severe behavioral changes.

Canine MPS IIIB is the only large mammalian model of this disorder. Initial characterization of the model and the normal and mutant DNA sequences have been reported. The disorder is seen in young adult dogs (2-3 years) as cerebellar ataxia. Clinical signs progress from their onset for 1-2 years, before animals require euthanasia. The mutation is an insertion of 45 A residues. It is critical to document the natural history of the disease in this model so as to facilitate evaluation of therapies. Herein, we report on the initial findings in young and aged affected dogs. Examination of tissues from 3-6 month-old affected dogs found distinct lysosomal storage in liver and kidney, but not in the CNS. Biochemical analysis of the CNS from dogs from 3 months to 5.6 years of age has been conducted. Analyses of cerebral grey matter found the proportion of GM2 and GM3 gangliosides (mole%) to have been increased 5 and 3 fold, respectively, compared to normal levels. Increases are evident as early as 3 months of age.

Canine MPS IIIB, an important resource to investigate pathogenesis and evaluate therapies, especially with regard to assessing both the treatment of a large brain as well as the efficacy and safety of therapy over years. Supported by the NIH (RR02512 and RR007063) and The National MPS Society, Inc.

Evidence for Altered Glutamine/Glutamate Synthesis in the SSADH-Deficient Mouse. *K.M. Gibson¹, G.M.I. Chowdhury², A.B. Patel², M. Gupta¹, H. Senephansiri¹, K.L. Behar².* 1) Molecular and Medical Genetics, Oregon Health & Science Univ, Portland, OR; 2) MRRC, Yale Univ, New Haven, CT.

Succinate semialdehyde dehydrogenase (SSADH) deficiency is a rare autosomal recessive inherited defect of GABA catabolism associated with increased gamma-hydroxybutyrate (GHB) and GABA. The murine SSADH knockout (KO) was created to study the pathophysiological mechanisms associated with the human disorder. Neurochemical studies have shown lower brain glutamine in affected patients and KO mice, suggesting altered astroglial/neuronal substrate trafficking. In this study, we infused the astroglial substrate, [2-¹³C]acetate to follow astroglial glutamine synthesis and repletion of neuronal glutamate/GABA in 17-day old KO and wildtype (WT) urethane-anesthetized mice. Mice were infused with [2-¹³C]acetate for 2 hrs (i.p.) using a bolus-variable rate infusion followed by freezing. Concentrations and ¹³C enrichments of amino acids were determined in extracts of cerebral cortex using ¹H-¹³C-NMR. Cortical GABA concentration was greater in KO mice (KO, 12.9 +/- 0.9; WT, 4.7 +/- 0.7 umol/g, P=0.001), whereas glutamate (KO, 9.7 +/- 0.6; WT, 11.3 +/- 1.2 umol/g, P<0.05) and glutamine (KO 3.4 +/- 0.3; WT, 5.7 +/- 0.3 umol/g, P=0.001) were lower. In [2-¹³C]acetate infused mice the steady-state % ¹³C enrichments of glutamine-C4 (KO, 11.2 +/- 0.9; WT, 20.7 +/- 1.6, P=0.001) and GABA-C2 (KO, 3.6 +/- 0.6; WT, 10.6 +/- 0.8, P=0.001) were lower in KO mice, although no differences (P>0.15) were observed for glutamate-C4 or aspartate-C3 enrichment. The combined effect of the lower concentration and ¹³C-enrichment in KO mice suggest that [4-¹³C]glutamine formation from [2-¹³C]acetate was decreased (67%). The steady-state level of [4-¹³C]glutamate was also lower (23 %), although [2-¹³C]GABA was similar between groups. Immunoblotting of glutamic acid decarboxylase isoforms (GAD65/GAD67) revealed a 50% reduction of both in hippocampus, cortex and cerebellum of KO mice as compared to WT animals. These in vivo data provide evidence for reduced astroglial glutamine metabolism in KO mice, yielding new insight into the neurologic abnormalities in both affected patients and KO mice.

Correlation between the age of onset/severity of Hereditary Inclusion Body Myopathy and polymorphisms within the dystroglycan gene. *E. Gottlieb*¹, *C. Ciccone*¹, *D. Darvish*², *S. Naiem*², *P. Savelkoul*¹, *D. Krasnewich*¹, *W. Gahl*¹, *M. Huizing*¹. 1) MGB, NHGRI, NIH, Bethesda, MD; 2) HIBM Research Group, Encino, CA.

Dystroglycan (DG), an integral protein in the Dystrophin-Glycoprotein Complex (DGC) codes for two proteins, and -DG. -DG is a membrane glycoprotein linking the membrane spanning -DG to the extracellular matrix and the cytoskeleton through non-covalent interactions. Functional domains in the DG protein include a transmembrane and dystrophin binding domain in the -DG region, and a mucin-like domain, rich in serine and threonine residues for potential O-linked glycosylation, in the -DG region of the protein. Defects in the DGC cause certain muscular dystrophies, but no human mutations in DG have been reported, despite the identification of many polymorphisms.

Hereditary Inclusion Body Myopathy (HIBM), an adult onset neuromuscular disorder characterized by progressive myopathic weakness and atrophy, caused by defects in *GNE*. *GNE* encodes UDP-GlcNAc epimerase/ManNAc kinase, the rate-limiting enzyme of sialic acid synthesis. We recently demonstrated hypoglycosylation of muscle -DG in HIBM patients, possibly caused by a deficiency of free sialic acid. Severity and age of onset of symptoms vary widely among patients, even those with similar *GNE* mutations and within the same family.

We studied correlations between DG-polymorphisms and symptoms in 32 HIBM patients, mainly of Iranian-Jewish descent. All patients carried C2725G in the 3'UTR, as well as three unreported amino-acid substituting polymorphisms: T488G (D163E), C741G (P248A), and T2611C (V871A), located outside of functional domains or glycosylation sites. Two subjects exhibited G1307A (T436T), located in a potential O-glycosylation site in a conserved area of the mucin-like domain. There was no correlation between this polymorphism and clinical symptoms; one patient had onset at age 31 and wheelchair-bound at age 40, while another had onset at age 22 and walks at age 26. More subjects are needed to draw definite conclusions. In addition, variations in other genes, like laminin-2, and 2,6-sialyltransferase, may influence clinical severity.

The role of metabolic disorders and trace elements in the pathogenesis and treatment of children with autism spectrum disorders. *J. Hou.* Dept Medical Genetics, Chang Gung Children's Hosp, Taoyuan, Taiwan.

The autism spectrum disorder (ASD) is a disease of complex genetic architecture. The underlying etiologies of ASD are heterogeneous and there is no effective treatment till now. When the metabolic consequences of disturbed biochemical pathways or trace elements are well defined, treatment with diet, drugs, or nutritional supplements may bring about a reduction in autistic symptoms. A prospective study evaluating evidence for metabolic etiologies in ASD, as well as for the efficacy of specific vitamin or drug treatments will be presented. We have screened 148 children (88 males, 60 females) who have pervasive developmental disorder with some autistic behaviors. CCDI (Chinese Child Developmental Inventory) study was also performed before and after medical treatment. One hundred normal samples have been prepared for biochemical and genetic comparison. ASD can be associated with fragile X syndrome and several metabolic disorders, therefore laboratory evaluation including tandem mass study for inherited metabolic diseases, and cytogenetic or/and molecular studies for Rett syndrome (MECP2 gene), chromosome anomalies, and fragile X syndrome were performed in each case. Several metabolic defects have been associated with autistic symptoms. These include lactic acidemia, methylmalonic acidemia, hyperphenylalaninemia, hypervalinemia and hyperleucinemia/isoleucinemia. Low blood zinc but high copper levels were found in 75 percent of the patients. The most common chromosomal anomalies were fragile X syndrome (n=15, 13/88 boys, and 2/60 girls). Other co-current anomalies included 47, XY, +m (inv dup 15) de novo (n=3), der(9)t(19;?)(q13.3;?) de novo, and del(2)(2p24). A 6~12-month treatment course with zinc gluconate, vitamin B6 and B12 in these patients showed significant improvement in cognition and social interaction by CCDI evaluation. Those work-ups including metabolic evaluation will distinguish ASD unequivocally from other developmental disabilities. Metabolic disorders and trace elements imbalance may play an important role of the pathogenesis and treatment of children with ASD.

Apoptosis in a mouse model for metachromatic leukodystrophy and the direct role for sulfatide. *J. Liu, C. Regan, T. Rupar.* biochemical Genetics Lab, Child & Parent Resource Inst, University of Western Ontario, London, ON, Canada.

Metachromatic leukodystrophy (MLD) is a progressive neurodegenerative disorder caused by a deficiency of arylsulfatase A (ASA) and is inherited in an autosomal recessive manner. MLD most frequently presents in late infancy and death occurs by about 6 years. ASA hydrolyses the glycolipid sulfatide which is primarily present in myelin. A deficiency of ASA results in the accumulation of sulfatide. The neuropathological characteristics include demyelination, gliosis and formation of metachromatic granules. There is atrophy and the reduction of granule and Purkinje cell numbers in the cerebellum. A knock-out mouse model (ASA KO) of MLD (Dr. V. Gieselmann) has lysosomal sulfatide storage of but does not show the gross white matter changes seen in humans. The mechanism by which sulfatide storage causes the neuropathology of MLD is not understood. The presence and number of cells dying in the murine cerebellum by apoptosis was studied in the ASA KO and control mice by observing DNA fragmentation using the TUNEL stain. The total number of apoptotic cells in mouse cerebellum from 1, 5, 10, 14, 21 and 28 days to 12 months was determined. ASA KO mice had significantly more apoptotic cells after 21, 28 days and 12 months with 50-70% more apoptotic cells in the cerebellum overall and about 80-180% more in white matter. The increase in the number of apoptotic cells was observed in the external germinal layer, molecular layer and internal layer. Sulfatide was tested for its ability to directly induce apoptosis by treating (6.25-25g/ml) primary neural cell cultures from both ASA KO and control mice in vitro with sulfatide or galactocerebroside as a control. Sulfatide induced morphological changes within 1-3 hr and increased the number of apoptotic cells. Galactocerebroside had no effect. Immunohistochemical staining of glial fibrillary acidic protein showed considerable drawing back of cellular processes most prominent in astrocytes. These data indicate that sulfatide may participate directly in the induction of apoptosis and the pathophysiology of MLD in the ASA KO mouse.

Arterial calcifications in adult cystinosis patients post-renal transplant. *K. O'Brien¹, M. Ueda¹, R. Kleta¹, I. Bernardini¹, D. Rosing², A. Ling³, D. MacAvey², W.A. Gahl¹.* 1) SHBG, MGB, NHGRI, NIH; 2) Cardiology Department, NHBLI, NIH; 3) Department of Radiology, NIH.

Cystinosis, an autosomal recessive disorder of lysosomal cystine accumulation, results from mutations in the CTNS gene that encodes the cystine transporter, cystinosin. Renal tubular Fanconi syndrome occurs in infancy, followed by rickets, growth retardation, hypothyroidism, and finally renal failure, which occurs at approximately ten years of age. Treatment with cysteamine decreases cellular cystine levels, retards renal deterioration, and allows for normal growth. For poorly treated patients, renal transplantation allows for survival at least into the third to fifth decades, but individuals experience other, non-renal complications of the disease. We reviewed routine chest and head CT scans on 27 post-transplant cystinosis patients. Thirteen of the 27 patients in our cohort had vascular calcifications, including 11 with coronary artery calcification. One 25-year old man required a 3-vessel coronary artery bypass graft surgery. The finding of vascular calcification was correlated with a history of renal disease, and having lived at least 20 years without cysteamine therapy. Three conclusions emerge from our experience in the long-term evaluation of cystinosis patients and their response to cysteamine treatment. First, cystinosis patients not chronically treated with a cystine-depleting agent experience progressive kidney disease culminating in end-stage renal disease, and the need for dialysis and renal allograft replacement therapy. Long-term oral cysteamine therapy starting in infancy can prevent or delay this outcome. Second, the metabolic disturbances that occur with chronic kidney disease, and with maintenance therapy for renal allografts, predisposes these patients to the premature development of coronary artery and other vascular calcifications. Third, adult post-transplant cystinosis patients should be screened for vascular calcification, which may be a useful non-invasive measure of atherosclerosis and, in some cases, end organ ischemia.

Mutations in genes coding for copper transporting ATP7A and ATP7B in Czech Republic. *L. Pospisilova¹, E. Flachsova¹, Z. Marecek², H. Hansikova¹, I. Halfarova³, R. Bruha², P. Fruhauf⁴, J. Zeman¹, P. Martasek¹.* 1) Department of Pediatrics and Center of Integrated Genomics, 1st School of Medicine, Charles University, Prague, Czech Republic; 2) Internal Medicine 4, 1st School of Medicine, Charles University, Prague, Czech Republic; 3) Department of Pediatrics, Municipal Hospital, Frydek-Mistek, Czech Republic; 4) Faculty General Hospital Prague, Department of Pediatrics, Czech Republic.

Copper plays an essential role in biology as a cofactor for many enzymes. There are two intracellular copper binding P-ATPases in human: ATP7A and ATP7B. Their genes are located on chromosomes Xq13.3 and 13q14.21. ATP7B deficiency causes autosomally-recessive inherited Wilson disease (WD), manifested by low serum ceruloplasmin and accumulation of copper in liver and in brain. The deficiency in ATP7A causes X-linked Menkes disease (MD), resulting in decreased intestinal absorption of copper, low copper levels in serum, and defective distribution of copper in tissues. We report here clinical, biochemical and molecular investigation in family with occurrence of Menkes disease. A boy with clinical symptoms of MD was referred to our hospital at the age of 4 months. Laboratory findings showed low serum copper and ceruloplasmin and low activity of Cu-dependent cytochrome c oxidase (COX) in thrombocytes. Molecular analysis revealed previously not published mutation in gene for ATP7A (Q724X). Mutated protein is truncated, therefore, its function is diminished. Molecular analysis allows genetic counseling and prenatal diagnosis in affected family. In the WD gene, ~ 200 different mutations have been identified. These findings, together with the large size of the gene, complicate the genetic analysis. However, in Central Europe, the H1069Q mutation seems to be present in 40-70 % of analyzed alleles. A group of 74 patients from the Czech Republic, diagnosed with WD, was screened for the H1069Q mutation in the WD gene by RFLP. 17 patients were homoallelic, 27 heteroallelic, and in 30 the H1069Q mutation was not detected. The frequency of mutated allele in our study group, 41 %, is in accordance with its occurrence in Central Europe. Supported by Grant # LN 00A079.

Investigating the role of glycerol kinase (GK) in insulin resistance by microarray analysis in a glycerol kinase knock out mouse. *L. Rahib*¹, *N. MacLennan*², *Z. Fang*^{3,4}, *S. Horvath*^{3,4}, *K.M. Dipple*^{2,3}. 1) Biomedical Engineering; 2) Pediatrics; 3) Human Genetics; 4) Biostatistics, UCLA, Los Angeles CA.

Glycerol kinase deficiency (GKD) is an inborn error of metabolism predisposing individuals to insulin resistance and diabetes mellitus (Gaudet et al., 2000). Peroxisome proliferator-activated receptor ligands are effective therapy for insulin resistance, and induce GK expression in adipocytes (Guan et al., 2002). The purpose of this study was to define the role of GK in insulin resistance by evaluating the changes in gene expression in a glycerol kinase (Gyk) knock out (ko) mouse model. Total RNA was extracted from brown fat from 2 day old Gyk ko and wildtype (wt) male mice. Microarray analysis (Affymetrix mouse 430) revealed 180 genes with differential expression (>1.2 fold) in the ko males compared to wt. Gene sorting demonstrated differential expression of genes in metabolism, transcription regulation, cell death/apoptosis, nucleic acid binding, DNA packaging, carbohydrate metabolism, and differentiation/regulation of proliferation. EASE analysis defined 51 groups with increased and 100 groups with decreased ($p < 0.05$) expression in Gyk ko mice. Expression levels of genes involved in lipid biosynthesis, fatty acid biosynthesis/elongation, glycerophospholipid/phospholipid metabolism were decreased ($p < 0.01$) in the ko mice. While expression levels of genes involved in transcription regulation, oxidoreductase activity, and protein sumoylation were increased ($p < 0.01$). Pathway Assist (Stratagene) analyses confirmed direct and indirect connectivity of GK and the genes involved in transcription regulation and lipid metabolism that were differentially expressed in the microarray. This confirms that GK deletion is integral with other metabolic and transcriptional networks. In conclusion, in a Gyk ko mouse, gene expression levels in categories including lipid metabolism and transcription are modified. Further evaluation of these genes will allow us to understand the role of GK in insulin resistance and diabetes mellitus. Studies of the network of glycerol metabolism in adipocytes will allow better understanding of the relationship between glycerol and glucose metabolism.

Carnitine metabolism in long-chain acyl-CoA dehydrogenase deficient mice. *P. Wood*¹, *N. van Vlies*², *L. Tian*¹, *H. Overmars*², *A. Bootsma*², *W. Kulik*², *R. Wanders*², *F. Vaz*². 1) Dept Genetics, Univ Alabama at Birmingham; 2) Departments of Clinical Chemistry and Pediatrics, Academic Medical Center, University of Amsterdam, The Netherlands.

We investigated tissue acylcarnitines and carnitine biosynthesis in wild type (WT) and long-chain acyl-CoA dehydrogenase deficient (LCAD^{-/-}) mice. We found C14:1 acylcarnitine in all tissues from LCAD^{-/-} mice. Accumulation of acylcarnitines (C9 and C11) in LCAD^{-/-} muscle was also observed, which most likely represents peroxisomal oxidation products of phytanic acid, confirming the role of LCAD in branched-chain fatty acid metabolism. Relevant levels of 3-hydroxy-acylcarnitines were found in heart, muscle and brain in WT mice, suggesting that in these tissues long-chain 3-hydroxyacyl-CoA dehydrogenase is rate limiting for mitochondrial -oxidation. The 3-hydroxy-acylcarnitines were absent in LCAD^{-/-} tissues indicating that, in this situation, the -oxidation flux is limited by LCAD. Additionally, a marked deficiency of acetylcarnitine was observed in LCAD^{-/-} hearts. Furthermore, we investigated the use of the carnitine precursor butyrobetaine (BB) for potential therapy. BB was provided in the water (400mg/L) for 2 weeks and mice were subjected to cold challenge. BB treated males showed greater cold tolerance, while females did not. LCAD^{-/-} mice, both sexes, were carnitine-deficient based on low carnitine levels in serum and heart. BB treatment restored serum carnitine levels in female LCAD^{-/-} mice but not in males, and in both sexes resulted in increased serum concentrations of the long-chain acylcarnitines (C14), indicating that BB induced carnitine production and/or enhanced acylation to carnitine. Despite increased serum carnitine levels in female LCAD^{-/-} mice, they remained cold intolerant. BB treatment in male LCAD^{-/-} mice did not restore carnitine levels but did significantly increase their cold tolerance. It appears that females handle BB differently than males, as shown in LCAD^{-/-} females with BB treatment, displayed high serum and tissue BB values (increased 2-7 fold), whereas the BB levels in male LCAD^{-/-} mice showed no change..

Molecular and biochemical testing for creatine transporter deficiency in an MR population suggests a 1% prevalence. *T. Wood¹, E.H. Rosenberg², A.J. Clark¹, L.S. Almeida², C. Jakobs², S.P. Young³, R.E. Stevenson¹, H. Taylor¹, M.J. Friez¹, C.E. Schwartz¹, G.S. Salomons².* 1) Greenwood Genetic Center, Greenwood, SC; 2) Department of Clinical Chemistry, VU University Medical Center, Amsterdam; 3) Division of Medical Genetics, DUMC, Durham, NC.

Creatine transporter deficiency is an X-linked genetic condition resulting from mutations in the SLC6A8 gene localized to Xq28. Lack of this transporter prevents cellular uptake of creatine and primarily affects normal cognitive function. Affected males present with mental retardation, speech delay, seizures and autistic behaviors. Carrier females may show mild learning problems. Diagnosis can be made via proton MRS studies, an elevated urinary creatine / creatinine ratio, or molecular analysis. A recent study in a European collection of males suspected of having X-linked mental retardation (XLMR) suggested the incidence of the disorder could be as high as 2%. To determine the incidence in a collection of males with non-fragile X mental retardation, we performed a retrospective DNA-based study on 501 males with mental retardation and a prospective urine-based study on 225 males with mental retardation of unknown cause. DNA sequencing of the SLC6A8 coding region identified seven potentially pathogenic mutations: 4 missense, 2 small deletions, and 1 splice site mutation. Testing in 600 normal males did not identify any of these changes. The prospective urine-based study was performed by stable isotope dilution tandem mass spectrometry. One patient out of the 225 males studied was identified with an elevated creatine / creatinine ratio and molecular analysis demonstrated a previously identified p.F408del mutation. A clinical review of all eight patients suggests that seizures can occur later in childhood and the disorder may be slowly progressive in nature. The observed overall incidence of the creatine transporter deficiency of 1.1% (8 / 726) among males with mental retardation of unknown cause is 3-10 times higher than other XLMR conditions except for the Fragile X syndrome and suggests that testing for creatine transporter deficiency should be considered in the initial work-up of males with mental retardation.

Altered splenic gene expression in mice and humans with Gaucher disease. *Y.H. Xu, L. Jia, B. Quinn, G.A. Grabowski.* Dept Pediatrics, Div Human Gen, Children's Hosp Medical Ctr, Cincinnati, OH.

Gaucher disease is an autosomal recessively inherited disease caused by mutations at the acid -glucosidase (GCase) locus (GBA). To understand the molecular pathophysiology of Gaucher disease, global gene expression was examined by microarray analyses of total splenic mRNAs from Gaucher mice and an affected patient. In mice, the progressive mRNA alterations were evaluated in the D409V/D409V point-mutated model. In these mice, RNA microarray analyses consistently identified that 5~9% of genes in inflammatory, signal transduction, transport processes and sphingoglycolipid metabolic pathways showed statistically significantly altered expression. Of these, 48% were up-regulated (2-24 fold) and 52 % were down-regulated (2-50 fold). Among the signal transduction and metabolic transport related genes 30-50% were differentially expressed (up to down or down to up) at the mid/late stages compared to the early stages. In sphingoglycolipid metabolic pathway, three genes [UDP-glucose ceramide glucosyltransferase (Ugcg), -galactosidase 1 (Glb1), and neuraminidase 1 (Neu1)] increased up to 5 fold with age. A similar pattern of mRNA expression was seen in a spleen sample from an adult human with non-neuropathic Gaucher disease. These patterns differed significantly from that in a human Niemann-Pick B spleen. These findings demonstrate a profound potentially characteristic change of gene expression in spleen of Gaucher disease by microarray analysis and suggest that the pathophysiology of GCase deficiency may involve the alteration of multiple gene expression as the disease progresses.

Osteopenia and osteoporosis: previously unrecognized features of Fabry disease. *D.P. Germain¹, K. Benistan¹, S. Perrot², C. Thevenot¹, S. Coulon¹, C. Mutschler².* 1) Dept Genetics, European Hosp Georges Pompidou, Paris, France; 2) Dept Radiology, European Hosp Georges Pompidou, Paris, France.

Background: Fabry disease (FD) is an X-linked inborn error of metabolism due to the deficient activity of alpha-galactosidase, a lysosomal enzyme. While the deposition of uncleaved glycosphingolipids throughout the body is known to have protean clinical manifestations, no data is available regarding the skeletal involvement.

Methods: We prospectively investigated bone involvement in 27 hemizygous males with a mean age of 32 years (range: 16-61 years) affected with classic FD. Bone densitometry was assessed in all patients, using dual energy X-ray absorptiometry (Hologic QDR-4500W).

Results: Bone densitometry examination revealed a statistically significant decrease in bone mineral density (BMD). Using the World Health Organisation (WHO) classification of BMD abnormalities (normal: T score > -1; osteopenia : -2.5 < T score < -1; osteoporosis: T score < -2.5), the following results were obtained at testing lumbar spine and femoral neck : T score normal at both sites : 11% (3/27); osteopenia at one site and normal T score at other site : 33% (9/27); osteopenia at both sites 19% (5/27); osteoporosis at one site and normal T score at other site: 4% (1/27); osteoporosis at one site and osteopenia at other site: 30% (8/27); osteoporosis at both sites 4% (1/27). Eighteen patients had normal glomerular filtration rate.

Discussion: This is the first study demonstrating that FD is associated to an increased risk of developing BMD abnormalities in male patients. Osteopenia and/or osteoporosis were detected in 89% (24/27) of our patients, even in the absence of renal insufficiency. With the emerging enzyme replacement therapies for FD and the hope of increased life expectancy, reduced BMD may become an important symptom to consider. We advise to perform bone densitometry in all FD patients as a baseline assessment in order to assess their risk of pathologic fracture.

ORAL DEFECTS OF BRAZILIAN CASES OF MUCOPOLYSACCHARIDOSIS. *A.C. Santos^{1,2}, A.C. Paula¹, T.P. Delboni¹, D.R. Bertola¹, L.M. Albano¹, L.A. Soares Jr², R. Giugliani³, C.A. Kim¹.* 1) Pediatrics, Instituto da Criança HC-FMUSP, So Paulo, So Paulo, Brazil; 2) Unidadede Odontologia do Instituto Central ,HC-FMUSP; 3) Servio de Gentica Mdica, Hospital de Clinicas de Porto Alegre, e Departamentos de Gentica e Bioqumica da UFRGS.

Mucopolysaccharidoses (MPS) are a heterogeneous group of lysosomal storage disorders caused by the deficiency of enzymes involved in degradation of glycosaminoglycans (GAGS). There are six recognized clinical types. Its pattern of inheritance is autosomal recessive, except for MPS II, which is X-linked. The course is progressive with multisystem involvement, specially skeletal, cardiac, pulmonary and abnormal facies including oral manifestations. We report on 5 Brazilian patients with MPS (1 MPS II, 1 MPS IIIC, 1 MPS IVA, 1 MPS VI and 1MPS VII) with abnormal oral findings. The mean age at diagnosis was 4y2mo (ranging from 1y9mo to 7y3mo) and their current age ranged from 6y to 11y4mo. Parental consanguinity was observed in 2/5 families (40%). All patients presented coarse facies, buccal respiration, limited opening of the mouth due to temporomandibular joint abnormality, drooling, lip enlargement, protruding tongue (macroglossia), hyperplastic gingivitis, ogival palate, abnormally wide teeth and flake enamel. Others findings: anterior open bite, spacing of the teeth, short mandible, dental caries (60%), dentigerous cysts, later eruption of the teeth (40%), sleep apnea, and bruxism (20%). This study alerts for the importance of oral manifestations in MPS. An adequate treatment of the structural and functional oral defects in different types of MPS is recommended by a team of health specialties, to improve the quality of life of these patients.

Correlation between interleukin 6 promoter gene polymorphism and stroke in patients with Fabry disease. G.

Altarescu¹, D. Moore², R. Schiffmann². 1) Genetic Dept, Shaare Zedek Medical Ctr, Jerusalem, Israel; 2) Developmental Metabolic Neurology Branch, NIH, Bethesda MD, USA.

Objective: To determine the incidence of the common polymorphism of interleukin (IL)-6 among patients with Fabry disease and ascertain the relationship to the occurrence of cerebral stroke in these patients. **Introduction:** Fabry disease is an X-linked recessive disorder associated with early onset stroke, marked by deep small infarcts as documented on perfusion- and diffusion-weighted magnetic resonance imaging (MRI). A correlation between the IL-6 plasma levels and presence of the 174GC promoter polymorphism with stroke in control populations has been previously shown. **Material and Methods:** Genotyping for the 174GC promoter polymorphism of IL-6 was performed in 51 hemizygotes male patients with Fabry disease and correlated with MRI evidence of white matter lesions. The polymorphisms in the IL6 gene were compared using ANOVA (both parametric and non parametric) for the three genotype groups (G/G, G/C and C/C). **Results:** None of the 9 patients (17.6%) with the G/G genotype had evidence of lesions; the G/C genotype was seen in 20 patients (39.2%) with a mean MRI load of 4.3811.15, and 22 patients had the C/C genotype (43.1%) with a mean MRI load of 355.26557.17; Thus, there was no significant difference between patients who had at least one G allele with regard to evidence of stroke, but both were significantly different from those with the C/C genotype ($p=.001$ and $.002$, respectively). **Conclusions:** The prevalence of the C/C genotype of the IL-6 promoter polymorphism among patients with Fabry disease is greater than in control Caucasian populations, and appears to carry a greater risk for developing white matter lesions on MRI. In that the pro-inflammatory cytokine IL-6 may be released shortly after hypoperfusion of the brain, the interesting question raised by the current study is whether the C/C genotype may be a modifier and/or marker microvascular abnormalities in Fabry disease.

Diffuse Traumatic Brain Injury-Induced Alteration of Mitochondrial DNA Copy Numbers in the Immature Rat Brain. *R. Bai*¹, *T. Chang*², *I. Cernak*³, *A.I. Faden*³, *L. Wong*¹. 1) Inst Molec & Human Genetics, Georgetown Univ Med Ctr, Washington, DC; 2) Neurology and Pediatrics, Children's National Medical Center, Washington, DC; 3) Neuroscience, Georgetown University Medical Center, Washington DC.

Mitochondrial gene expression in liver, heart or skeletal muscle is known to change in response to oxidative stress and age. A new developmental model of diffuse traumatic brain injury (TBI) indicates a difference in bioenergetic response to injury in the early immature brain. We propose to examine this response by evaluating mitochondrial biogenesis in the early immature rodent brain after diffuse TBI. Total genomic DNA was isolated from 7 day old male Sprague Dawley (Harlan) rat brain. The rat pups were injured and sacrificed at 4 and 24 hrs after injury and compared to naives. The mitochondria DNA (mtDNA) copy number for each sample was determined by real-time quantitative PCR (RT qPCR) and normalized to beta 2 microglobulin gene, a single copy nuclear gene. The cortex and subcortex reflect a higher mtDNA copy number. In all regions of the brain examined (cortex, subcortex, hippocampus and cerebellum), the mtDNA copy number decreases acutely 4 hrs post-injury (437 31; 251 31; 192 3 and 10 2; respectively) compared to naives (603 2; 450 98; 278 59; and 73 32). However, by 24 hrs after injury there is an increase (1207 83; 536 46; 217 9; and 97 1) suggesting a compensatory amplification. The cerebellum is most affected at 4hrs post-injury while the cortex has the greatest amplification response at 24hrs post-injury. These results demonstrate that there is an active mitochondrial biogenetic response to diffuse TBI which occurs acutely.

Evaluation of Adrenal Function in Asymptomatic Boys with X Linked Adrenoleukodystrophy Background. *P. Dubey, H.W. Moser, L. Keldiyarova, A. Fatemi, R.O. Jones, L. Bezman, G.V. Raymond.* Neurology, Kennedy Krieger Institute, Johns Hopkins University, Baltimore, MD.

Background: X Linked Adrenoleukodystrophy (X-ALD), is commonly associated with adrenal insufficiency that can be treated to prevent life threatening complications. However, Not many studies have undertaken detailed evaluation of adrenal function in X-ALD. **Methods:** 89 X-ALD boys were studied as a part of a prospective evaluation of Lorenzo's Oil therapy. Adrenocorticotropic Hormone (ACTH) Stimulation test was administered unless known history of Adrenal Insufficiency was present. Baseline ACTH and Cortisol was measured in all patients. Chi2 test of homogeneity used for statistical analyses. **Results:** Mean (SD) baseline age (yrs) was 5.45(4). The Mean (SD) follow-up (yrs) was 4.46(3.01). 88% of X-ALD boys had adrenal insufficiency by last visit, 90% of these were diagnosed with adrenal insufficiency by 10 yrs of age. 85% had baseline adrenal insufficiency, 3% developed it during follow-up. 77% patients first came to attention due to an affected family member, 23% presented with adrenal insufficiency and were subsequently diagnosed with X-ALD. Mean (SD) age of onset of adrenal insufficiency was 4.38(3.59) years, ranging from 8 months to 13 years. Mean (SD) Serum basal Cortisol and ACTH at baseline were 13.69 (6.38) micrograms/dl and 1106.84 (1503) picograms/ml respectively. Basal ACTH was elevated in 37% patients with normal response to ACTH stimulation and normal Basal Cortisol. Greater percentage of patients had normal Basal Cortisol levels in the presence of abnormal ACTH stimulation test and abnormal basal ACTH (93% vs. 6%, $p=0.001$). **Conclusion:** Addison's disease is a prominent cause X-ALD identification besides family screening and occurs early in disease course. Baseline Cortisol may be normal with abnormal ACTH stimulation test, suggesting Addison's Crisis may be precipitated during stress, hence Baseline Cortisol alone is not a good marker of adrenal insufficiency. Patients may maintain normal response to ACTH stimulation test with compensatory increase in Basal ACTH masking clinical expression of adrenal insufficiency, hence require regular and careful monitoring.

Gaucher genotype N188S/RecNciI is associated with myoclonic epilepsy. *L. Kowarz*¹, *O. Goker-Alpan*¹, *S. Banerjee-Basu*², *L. Kinlaw*¹, *R. Schiffman*³, *A.D. Baxevanis*², *E. Sidransky*¹. 1) NSB, NIMH/NHGRI; 2) GTB, NHGRI; 3) DMNB, NINDS; NIH, Bethesda, MD.

Gaucher disease (GD), the inherited deficiency of lysosomal glucocerebrosidase, presents with a wide range of symptoms of varying severity. Significant genetic heterogeneity has been found in a subgroup of patients with type 3 chronic neuronopathic GD who develop progressive myoclonic epilepsy. These patients initially exhibit slowed horizontal saccades, but develop dementia, ataxia and spasticity. The records of 17 such patients were reviewed and 4 ethnically diverse subjects (2M: 2F) with genotype N188S/RecNciI were identified. One patient developed myoclonic jerks at age 5, the others developed myoclonus and then generalized seizures during their teens, and all exhibited prominent ataxia and EEG abnormalities consistent with a global cerebral process. One patient suffered from cognitive decline and one had learning disabilities. In two, the onset of myoclonic seizures preceded the diagnosis of GD. All had mild systemic manifestations of GD; one underwent splenectomy and one received enzyme replacement therapy.

The N188S mutation is rare and has been described as a mild allele producing high residual enzymatic activity. The RecNciI allele is presumed to be a null allele. Molecular modeling using the three-dimensional crystal structure of glucocerebrosidase demonstrated that mutation N188S is located in domain III of glucocerebrosidase, the region containing the TIM barrel forming the catalytic domain. This region is situated between strand 3 and helix 3, and the substitution of a serine does not alter surface charge distribution. N188S, V394L and G377S, all rare mutations associated with myoclonic epilepsy, are located on the outer edge of the active site of the enzyme and, hence, could cause similar alterations in protein function. The association of mutation N188S with the rare myoclonic phenotype in Gaucher disease suggests that N188S might alter local protein structure, binding, and/or post-translational processing, leading to neuronal cell death; it could also have a modifying role on other proteins involved in the pathogenesis of myoclonic epilepsy.

Impaired mitochondrial glutamate transport in autosomal recessive neonatal myoclonic epilepsy. *F. MOLINARI¹, A. RAAS-ROTHSCHILD², M. RIO¹, G. FIERMONTE³, L. PALMIERI³, F. PALMIERI³, N. KHADOM¹, A. MUNNICH¹, P. RUSTIN¹, L. COLLEAUX¹.* 1) INSERM U393, Hopital Necker-Enfants Malades, PARIS, France; 2) Dept of Genetics, Hadassah University Medical Center, Jerusalem, Israel; 3) Dept of Pharmaco-Biology, University of Bari, Bari, Italy.

Early myoclonic encephalopathy (EME) with suppression burst (SB) is an early onset malignant epilepsy syndrome characterized by a burst-suppression pattern of the EEG with bursts of spikes, sharp waves and slow waves, which are irregularly intermingled and separated by periods of electrical silence. The prognosis for EME is poor with no effective treatment and children either die before 2 years of life or survive in a persistent vegetative state. The lack of consistent neuropathological features suggests that etiology may vary from case to case. EME has been reported in nonketotic hyperglycinemia (MIM 605899), propionic acidemia (MIM 606054) and some malformative disorders. However, in most cases, the underlying mechanism of these disorders remains unknown.

Here we report on genetic mapping of an autosomal recessive EME to chromosome 11p15.5 and identification of a missense mutation (Pro 206 Leu) in the gene SLC25A22 (GC1) encoding one of the two mitochondrial glutamate symporters. The mutation co-segregated with the disease and altered a highly conserved amino-acid. Functional analyses showed that glutamate oxidation in patient cultured skin fibroblasts was strongly defective. Further studies in reconstituted proteoliposomes showed defective [14C]glutamate uniport and [14C]glutamate/glutamate exchange by mutant protein.

Intact glutamate metabolism is crucial for amino acid synthesis and oxidation, for ureogenesis and for normal function of the CNS. The identification of mutant GC1 as an etiology of EME emphasizes the unexpected importance of the mitochondrial component of glutamate metabolism in normal brain function, thus opening a novel field in the pathophysiology of EME.

Autism spectrum manifestations in cerebral folate deficiency. *P. Moretti¹, S. Peters², K. Hyland³, T. Bottiglieri³, R. Hopkin⁴, E. Peach⁴, B. Roa¹, C. Bacino¹, F. Scaglia¹.* 1) Mol and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Pediatrics, Baylor College of Medicine, Houston, TX; 3) Inst of Metab Disease, Baylor Univ Med Ctr, Dallas, TX; 4) Human Genetics, Cincinnati Childrens Hosp Med Ctr, Cincinnati, OH.

Intracellular folates participate in essential one-carbon transfer reactions. Progressive neurological dysfunction has been described in adults and children with an isolated deficiency of folate in the central nervous system. We report the clinical manifestations and biochemical features of 4 girls and 1 boy with cerebral folate deficiency (CFD). All patients had low cerebrospinal fluid (CSF) levels of 5-methyltetrahydrofolate, the biologically active form of folates in CSF and blood. Red blood cell folate, serum folate, and total plasma homocysteine were normal. All children exhibited psychomotor retardation and seizures with variable response to folinic acid. Three females had manifestations evocative of Angelman syndrome (AS) including facial features, ataxia, and hand flapping. AS DNA methylation studies and *UBE3A* mutation analysis were normal. The fourth female exhibited features of Rett syndrome in the absence of *MECP2* mutations or deletions. Through clinical observations, 4 of these patients demonstrated considerable repetitive behaviors, decreased eye gaze and communication deficits that warranted a formal evaluation for autism using the Autism Diagnostic Observation Schedule and the Autism Diagnostic Interview - Revised. Of the 2 patients formally evaluated thus far (2 children with AS-like features), both met criteria for autism. Regardless of their overall cognitive abilities, these children demonstrated deficits in communication and socialization that mirror those observed in children with idiopathic autism. Formal evaluations are currently being completed with the 2 other females. These findings demonstrate that children with autism and features of AS or Rett syndrome may have abnormalities of CSF folate, expanding the clinical spectrum of CFD. Future studies will determine the general relevance and frequency of these findings and elucidate the molecular mechanisms of reduced CSF folate in these patients.

Dysregulation of the neuronal actin cytoskeleton in identical twins with dystonia as a consequence of a bioenergetic defect. V. Procaccio^{1,2}, M. Gearing³, S. Ono³, CA. Gutekunst⁴, JL. Juncos⁴, A. Davila¹, BH. Wainer³. 1) MAMMAG, Univ California, Irvine, Irvine, CA; 2) Department of Pediatrics, University of California, Irvine, California; 3) Department of Pathology and Laboratory Medicine, Emory University, Atlanta, Georgia; 4) Department of Neurology, Emory University, Atlanta, Georgia.

Actin is a major cytoskeletal protein in neurons, and the dynamics of its assembly affect many aspects of cell motility and membrane turnover, representing a major ATP-consuming process in cells. Actin is co-transported with actin-binding proteins, including ADF and cofilin, that are essential for rapid turnover of actin filaments in vivo and regulated through a phosphorylation process. Recently cofilin has been shown to be translocated into mitochondria, crucial for the initiation of apoptosis. We reported extensive actin aggregation in the brains of identical twins with generalized dystonia and deafness associated with multiple developmental abnormalities. Immunohistochemistry revealed that rod-like inclusions in neocortex and spherical inclusions in basal ganglia were strongly immunoreactive with antibodies to ADF/cofilin. Two-dimensional electrophoresis of brain extracts demonstrated increased phosphorylation of cofilin. DNA microarray studies of mitochondrial gene expression using our Mitochip and real-time quantitative PCR in brain tissues showed increased expression of several genes including actin, cofilin, genes involved in apoptosis and mitochondrial ROS scavenging enzymes in response to increased mitochondrial ROS production. Expression of genes involved in mitochondrial ATP production was down-regulated in brain cortex. Sequencing of the entire mtDNA showed no abnormalities, thus pointing to a nuclear defect in these cases. Because actin reorganization requires ATP consumption, a defect in mitochondrial bioenergetics may perturb cellular ATP levels and alter the phosphorylation state of cofilin. Rod formation in response to mitochondrial stress may be a common pathophysiologic mechanism to protect neurons and conserve ATP by slowing actin filament dynamics.

Fabry disease in children. *M. Ries*¹, *S. Gupta*¹, *D.F. Moore*², *V. Sachdev*³, *J.M. Quirk*¹, *G. Murray*¹, *D.A. Rosing*³, *C.H. Robinson*¹, *E. Schaefer*⁴, *A. Gal*⁴, *J.M. Dambrosia*⁵, *S.C. Garman*⁶, *R.O. Brady*¹, *R. Schiffmann*¹. 1) DMNB/NINDS/NIH, Bethesda, MD; 2) University of Manitoba, Canada; 3) NHLBI/NIH Bethesda, MD; 4) University of Hamburg, Germany; 5) NINDS/NIH, Bethesda, MD; 6) NIAID/NIH, Rockville, MD.

Background: Fabry disease is an under-diagnosed, X-linked multisystem disorder. **Objectives:** To provide quantitative natural history data and novel clinical endpoints for therapeutic trials. **Methods:** We conducted a prospective, cross-sectional case-control study of 25 hemizygous children with Fabry disease (mean age 12.3 years) at the NIH. Main outcome measures were quality of life (Child Health Questionnaire), and sweating as assessed by quantitative sudomotor axon reflex test (QSART). **Results:** Quality of life: Pain scores in patients under 10 years of age were similar to those of a group of children with juvenile rheumatoid arthritis. The mean scores for Bodily Pain and Mental Health (patients 10 years) as well as Bodily Pain (patients 10 years) were lower than controls. QSART: Mean sweat volume in the Fabry group was significantly decreased compared with controls. Renal function, urinary protein excretion, cardiac function and structure were normal in the majority of patients. Patients with over 1.5 percent of normal residual -galactosidase A activity were free of cornea verticillata and had normal Gb3 values in serum and urine. All other children had Gb3 levels comparable to adult patients with Fabry disease. Acroparesthesia and cardiac abnormalities were generally present prior to anhidrosis and proteinuria. Crystallographic mapping of missense mutations identified a peripheral localization in individuals with residual enzyme activity. Mutations associated with left ventricular hypertrophy were localized in proximity to the catalytic site of the enzyme. **Conclusions:** Despite absence of major organ involvement, Fabry disease is already a burdensome disorder in childhood. We have identified important correctable (QSART, quality of life measurement) and preventable outcome measures for future therapeutic trials. Prevention of secondary complications in major organs will be the goal for long-term specific therapy.

Genetic and biochemical features of 18 patients with Pompe Disease. *T. Podskarbi*¹, *H.H. Goebel*², *B.G.H. Schoer*³, *H. Lee*⁴, *Y.S. Shin*⁴. 1) Molecular Genetics and Metabol, Munich, Bavaria, Germany; 2) Dept. of Neuropathology, University of Mainz, Germany; 3) Dept. of Neurology, University of Munich, Germany; 4) University Childrens Hospital, Munich, Germany.

Glycogen storage disease type II (Pompe disease) is caused by deficiency of lysosomal α -glucosidase (GAA), resulting in impaired degradation and lysosomal accumulation of glycogen. The GAA gene has been mapped to chromosome 17q25.2-25.3. Lesions in the gene lead to complete or partial inactivation of the enzyme. The early-onset form of the disease manifests shortly after birth and presents with progressive and generalized muscle weakness. Cardiac and respiratory insufficiency leads to death before 2 years of age. Patient with childhood and juvenile/adult form of the disease present with skeletal muscle weakness and respiratory failure later in life, although some of them show cardiac enlargement. In this report we have comprised biochemical parameters in relation with mutation data in a total of 18 German and Turkish patients (10 females and 8 males), 6 infantile, 2 childhood, 5 juvenile and 5 adult patients. Patients with childhood and juvenile/adult form show a various degree of a residual GAA activity in muscle, fibroblasts and in leukocytes in contrast to the infantile form. Except in one patient with the childhood form we were able to identify mutations of the GAA gene in both alleles of all patients. 7 novel mutations, A237V in exon 4, G293R in exon 5, L355P in exon 6, IVS6-2A>G in intron 6, H568L in exon 12, A611V and R620Q in exon 13 were detected. Among German patients, IVS1-13T>G in intron 1, 525delT in exon 2 and delExon18 are most common, and among Turkish patients c.2741AG>CAGG was most prevalent. The spectrum of the mutations can be correlated with the GAA activity expressed, which again reflects the clinical severity of the disease.

Clinical, biochemical and molecular heterogeneity of branching enzyme deficiency (Andersen disease). *Y.S. Shin¹, M. Vorgerd², H. Lee¹, T. Podskarbi³*. 1) Dept. of Pediatrics, University Childrens Hospital, Munich, Germany; 2) Dept. of Neurology, University Hospital, Bochum, Germany; 3) Molecular Genetics and Metabolism Laboratory, Munich, Germany.

Glycogen storage disease type IV known as Andersen disease or amylopectinosis, is a rare autosomal recessive disorder caused by deficiency of glycogen branching enzyme. The gene for this enzyme is located on chromosome 3q14. Although all forms of GSD type IV are due to aberration of this gene, but their clinical manifestation is extremely variable. The classic form, a severe liver form is characterized by hepatosplenomegaly and progressive hepatic fibrosis in the first 18 months of life, which usually leads to death before the age of 5 years. Patients with a rare nonprogressive variant (a mild liver form) do not develop cirrhosis and survive until adulthood. Patient with the infantile neuromuscular form of the disease may present at birth with severe hypotonia, muscle atrophy, and neuronal involvement resulting in death during neonatal period. In addition to a severe multisystem neonatal form, there are the juvenile form with myopathy and/or cardiomyopathy, an adult neuromuscular form of polyglucosan body disease (APBD) as well as an isolated skeletal muscular form with adult onset have also been reported. The branching enzyme activity is deficient in leukocytes, liver and cultured fibroblasts of the neonatal and infantile liver forms as well as of the cardiac form. About a 10% residual activity can be found in leukocytes among patients with APBD and the mild liver forms. Isolated muscle forms of GSD IV on the other hand show the deficient activity in muscle only and a normal activity in other tissues such as leukocytes, fibroblasts and liver. The molecular study of a German female with APBD revealed a compound heterozygosity for two missense mutations in the branching enzyme gene (R515H, R524Q) and of a Polish boy with the mild liver form for H528R and IVS5+2t>c. Further biochemical and molecular studies of various forms are necessary to understand the marked heterogeneity of the disease.

Mutated *AMN* causes Imerslund-Grasbeck syndrome in a canine model. *Q. He*¹, *M. Madsen*², *E.I. Christensen*², *P. Højrup*³, *H. Vorum*², *S.M. Tanner*⁴, *A. de la Chapelle*⁴, *S.K. Moestrup*², *J.C. Fyfe*¹. 1) Michigan State University, East Lansing, MI; 2) University of Aarhus, Denmark; 3) Odense University, Denmark; 4) Ohio State University, Columbus, OH.

Selective malabsorption of cobalamin (vitamin B₁₂) accompanied by proteinuria, known as Imerslund-Grasbeck syndrome or megaloblastic anemia 1 (I-GS, MGA1; OMIM 261100), is a rare autosomal recessive disorder. In Finnish kindreds, I-GS is caused by mutations in the cubilin gene (*CUBN*), located on human chromosome 10. However, not all patients have *CUBN* mutations; the amnionless gene, *AMN*, which is located on chromosome 14, has been recently identified as another I-GS-responsible gene. Functional study shows that cubilin and AMN forms a tight complex, cubam, to function as a cobalamin-intrinsic factor receptor on the apical membrane of intestinal epithelial cells. We have previously reported a canine I-GS model mapped to a region on dog chromosome 8 that exhibits conserved synteny with human chromosome 14q. We cloned the cDNA of dog *AMN* and found a 33bp in-frame deletion in the *AMN* gene of the affected dogs, by both RT-PCR and genomic PCR. The mutation segregates with the disease in the dog family and is absent in 110 unrelated normal dogs. Deletion of 11 amino acids abolished a putative transmembrane domain, which is potentially important for AMN to anchor on the apical membrane. We also developed antibody against dog AMN and found the AMN protein absent in affected dogs, indicating that the mutant AMN is secreted out of the cells or degraded inside the cells. Thus, canine I-GS is a homologue of one form of the human disease, and provides a novel animal model to study the molecular mechanism of cobalamin absorption, and in a broad sense, receptor biology.

Phenotypic variation among 3 Fabry pts with D231G mutation in a Korean kindred. *H.J Kim¹, H.E. Yim², J.G. Kim³, H.S. Kim⁴.* 1) Medical Genetics, Ajou Univ Col Medicine, Suwon, Korea; 2) Pathology, Ajou Univ Col Medicine, Suwon, Korea; 3) Radiology, Ajou Univ Col Medicine, Suwon, Korea; 4) Nephrology, Ajou Univ Col Medicine, Suwon, Korea.

Fabry dis. is an X-linked lysosomal storage disorder due to α -galactase A deficiency which results in accumulation of glycosphingolipid and leads to multisystem dis. Most of Fabry dis is found to have private mutations in GLA gene and no general correlation between genotype and clinical phenotype has been found to date. We report a Korean family, in which marked phenotypic variation was found among 3 members with D231G mutation. Proband, 37y.o male with end-stage renal disease, was found to have deficient α -galactosidase A activity and D231G mutation of GLA gene. Subsequently, identical mutation was found in two other members of the family; his mother, 62 y.o female who denied any subjective symptoms were found to have proteinuria(2+) and hematuria(2+) and 35y.o asymptomatic brother, showed occasional proteinuria(trace). Renal biopsy of proband showed marked glomerular sclerosis, global or segmental, vacuolated podocytes in non-sclerotic segments, interstitial fibrosis and infiltration of vacuolated histiocytes, tubular atrophy with some vacuolar cytoplasmic change of tubular cells and vascular sclerosis. Renal biopsy of his mother showed mesangial expansion and segmental sclerosis with thickening of capillary walls. Renal biopsy of his brother revealed prominent vacuolar podocytes in glomeruli without glomerular sclerosis or interstitial fibrosis. Characteristic myeloid bodies were easily found in podocytes and tubular epithelial cells. None of three cases showed angiokeratoma, a well recognized clinical characteristics of the dis., however, skin biopsy of the proband showed osmophilic laminated bodies in dermal fibroblasts and endothelial cells, but those of his brother and mother showed no such findings. Identification of mutation in a Fabry dis. pt. should be followed up with pedigree analysis and genetic counselling, in order to provide early Dx. for unrecognized at risk family members, which will lead to early therapeutic intervention for better clinical outcome.

An international, multicenter, longitudinal observational study evaluating the natural history of Mucopolysaccharidosis II (Hunter syndrome). *A. Kimura¹, M. Beck², R. Giugliani³, J. Fletcher⁴, J. Hopwood⁴, Y. Suzuki⁵, E. Wraith⁶, A. Conway¹, J. Lieb¹, S. Hirani¹, J. Muenzer⁷.* 1) Clinical Affairs, Transkaryotic Therapies, Inc, Cambridge, MA, USA; 2) University of Mainz, Mainz, Germany; 3) Hospital de Clinicas de Porto Alegre, Porto Alegre, Brazil; 4) Womens and Childrens Hospital, Adelaide, Australia; 5) Gifu University School of Medicine, Gifu, Japan; 6) Royal Manchester Childrens Hospital, Manchester, UK; 7) University of North Carolina, Chapel Hill, NC, USA.

Mucopolysaccharidosis II (MPS II or Hunter syndrome) is an X-linked recessive disorder caused by the deficiency of the lysosomal enzyme iduronate-2-sulfatase (I2S). MPS II is a progressive, multisystem disorder resulting from the accumulated storage of glycosaminoglycans (GAG). There is no effective therapy for MPS II; however, the safety and efficacy of I2S enzyme replacement therapy (ERT) as a treatment for MPS II is currently being investigated in clinical trials. The literature only contains case studies or data on small groups of patients. To gain a better understanding of the natural history of this disease, a 2-year, multicenter, observational study was initiated. This study will enable a comparison of the progression of the disease in untreated patients vs. patients receiving ERT in the future. Evaluations being performed in the study include medical history, physical examination, hearing and vision testing, ECG, MRI (cerebral & abdominal), urine GAG measurement, joint mobility assessment, pulmonary function tests, 6-minute walk test, intelligence and developmental testing, and an assessment of quality of life. Data will be collected at baseline and at 1 & 2 year visits for each patient. One hundred and eighteen MPS II patients have been enrolled. These patients had a mean age of 11.2 years at enrollment and represented the full range of disease severity. Patient demographics as well as baseline and 1 year data from the study will be presented. This prospectively collected clinical data will provide for the first time quantitative information on the degree and rate of disease progression in a lysosomal storage disorder.

Small neonatal head circumference - A previously unrecognized manifestation in Niemann-Pick disease type C that is useful in a diagnostic score. *E. Passarge*¹, *D. Schmidt*^{1,2}. 1) Institut für Humangenetik, Universitätsklinikum Essen, Essen, Germany; 2) University Children's Hospital Zürich, Switzerland.

Objectives: Niemann-Pick disease type C (NPC, MIM 257220), a rare, progressive autosomal recessive neurovisceral disorder due to mutations in either the NPC1 gene or the HE1 gene, respectively, was investigated with respect to early manifestations. Based on the new observation of a small neonatal head circumference a diagnostic score to facilitate an early diagnosis was developed. **Methods:** During a retrospective study of 21 patients the development and clinical course of NPC were examined. The diagnosis of NPC was based on the range of clinical manifestations, the course of illness, histochemical and biochemical data, and in four patients on mutation analysis. Based on a new observation, a reduced head circumference at birth, a diagnostic score (NPC score) was developed, including four other clinical signs of NPC, such as hepatosplenomegaly, prolonged icterus, and muscular hypotonia. The score was applied to patients and compared to an age-matched control group. **Results:** NPC was confirmed in all patients by clinical and laboratory data, and in four patients by molecular genetic analysis. A reduced neonatal head circumference at or below the 3rd percentile was found in 9 of 17 patients with the neonatal and infantile form of NPC compared to an age-matched control group. These patients showed scores indicative of NPC, in contrast to the control group. **Conclusions:** Reduced neonatal head circumference is a hitherto unrecognized manifestation of NPC. Integrated into the known clinical manifestations, we developed a diagnostic score (NPC score) which could facilitate an early diagnosis and avoid unnecessary gene tests.

Novel mutations in the ATP2A2 gene in two families with Darier's disease. *L. Peleg¹, M. Karpati¹, B. Goldman¹, B. Amichai².* 1) Genetic Inst, Sheba Medical Ctr, Ramat Gan, Israel; 2) Dept of Dermatology, Huzot Clinic of Clalit Health Services, Ashkelon, Israel.

Darier's disease (DD) is an autosomal dominant skin disorder characterized by loss of adhesion between epidermal cells and abnormal keratinization. The disease has a world wide distribution with a varied prevalence. Defects in the sarco/endoplasmic reticulum Ca⁺⁺ ATPase type 2 underlie DD. The encoding gene ATP2A2 is positioned on chromosome 12q23-24 and has 21 exons. To date 140 mutations had been identified in familial and sporadic cases of DD. The aim of this study was to identify the genetic defect underlying patients of two Jewish families of Eastern European ancestry: one family included an affected mother and pre-symptomatic healthy twins and the other comprised of six patients out of seven family members. Genomic DNA was extracted from peripheral blood and PCR was carried out to amplify the exons and flanking intron regions of the ATP2A2 gene which were subsequently sequenced. Two novel mutations were identified, one in each family. C391 to T (exon 5), altering arginine 131 to stop codon (R131X) and a change of A530 to C (exon 6) replacing glutamine 177 by proline. Both mutations resulted in restriction site elimination: Taq I and Bsr I for R131X and Q177P respectively. None of the mutations was present in 50 healthy individuals of the same ethnic origin. The mutations are located in the cytoplasmic beta-strand domain which functions as transduction site and is highly conserved.

Longitudinal neuropsychologic and neurologic findings in a mild form of Sly syndrome (mucopolysaccharidosis type VII). *E. Shapiro¹, K.J. Bjoraker¹, L. Charnas¹, S. Simonton², C. Whitley¹*. 1) Dept Pediatrics, Univ Minnesota, Minneapolis, MN; 2) Children's Hospitals and Clinics, Minneapolis, MN.

Sly syndrome, or mucopolysaccharidosis (MPS) type VII, is the rarest of the MPS disorders and results from beta-glucuronidase deficiency. Review of the extant case literature suggests a severe infant form typically presenting with hydrops fetalis and poor outcome, and juvenile forms; a mild form with little disability, and an intermediate form with mental retardation with decline in cognitive function in some cases. **Methods:** When a newborn male infant presented with jaundice and hepatomegaly, a subsequent bone marrow biopsy demonstrated large, toluidine blue inclusions in macrophage cells leading to the early diagnosis of MPS VII. A female sibling was also found to have this condition. We present longitudinal data on the girl with a very mild form and boy with mild mental retardation and somatic abnormalities. Biochemical, genetic, neurologic, neuroimaging, and neuropsychologic follow-up data were collected. **Results:** Physical examination of the boy exhibits mild skeletal abnormalities (e.g., scoliosis), small masses on the bottom of one foot, and segmental hairy nevi. The girl's examination is normal. The boy, age 8 and 13 at evaluations, has stable but impaired IQ (64 and 56 respectively), average face perception, motor coordination, verbal fluency, receptive vocabulary, and behavior; below average attention, achievement, memory, spatial perception, and gross motor coordination. The girl, age 6 and 11 at evaluations, has stable and normal IQ (84 and 92 respectively), average achievement, memory, perception, language, and behavior, and below average attention span, and fine motor coordination. Neurologic and imaging studies were essentially normal for both except effacement of the cervical cord (female) on follow-up. **Conclusions:** No neurodegeneration was noted. These children are not concordant in their phenotype; one mild and one impaired but stable. Their profiles show a familial mild executive dysfunction. Molecular genetic abnormalities (currently in progress) may prove helpful in distinguishing such mild forms of MPS VII from those with other, more severe prognosis.

Presentation of Previously Undiagnosed Inborn Errors of Metabolism to Emergency Departments. *D.L.*

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Background: Knowledge of characteristics of patients with undiagnosed inborn errors of metabolism (IEM) who present to the emergency department (ED) is critical for appropriate evaluation and often lifesaving management, and may be valuable for assessment of newborn screening programs. **Objective:** To determine whether patients with previously undiagnosed IEMs present to EDs prior to diagnosis and if so, whether they have history, signs and symptoms that should alert the ED physician to the possibility of IEM. **Design/Methods:** Descriptive retrospective chart review. Patients with clinically diagnosed IEM who live in New England were identified by geneticists involved in their care. ED visits and hospitalizations prior to IEM diagnosis were reviewed. **Results:** Information was available on 41 clinically diagnosed patients. Diagnoses included 14 different IEMs, most commonly fatty acid oxidation defects (41%) and propionic acidemia (24%). 30/41(73%) had ≥ 1 ED visit prior to diagnosis, 9/30 (30%) had ≥ 2 visits, up to 4/patient, total 45 visits. Median age at diagnosis was 4.5 mo (3 d to 5.2 yrs). Presenting symptoms, although consistent with IEM, were nonspecific, most commonly lethargy, seizures, tachypnea, apnea, cyanosis, hypothermia, poor feeding, vomiting. History is often remarkable for poor feeding, morning lethargy, developmental delay, hypotonia. Hypoglycemia, acidosis, hyperammonemia, elevated liver transaminases, elevated BUN were common laboratory abnormalities. Critical laboratory studies for evaluation of possible IEM were often not obtained in the ED. Most patients had multiple consults by nongenetic subspecialists prior to evaluation by a geneticist. **Conclusions:** The ED is a site of presentation of the majority of patients with undiagnosed IEM. The possibility of IEM is suggested by history and abnormal laboratory results. ED physicians, as well as primary care physicians and nongeneticist consultants, should be educated regarding recognition and evaluation of patients with possible IEM.

Brain magnetic resonance imaging analysis of a hypomorphic Smith-Lemli-Opitz Syndrome mouse model. *L.S. Correa-Cerro*¹, *J.P. Munasinghe*², *C.A. Wassif*¹, *F.D. Porter*¹. 1) Heritable Disorders Branch, NICHD/NIH, Bethesda, MD; 2) MRI Mouse Imaging facility/NIH, Bethesda, MD.

The Smith-Lemli-Opitz syndrome (SLOS) is a multiple malformation syndrome due to mutation of the 7-dehydrocholesterol reductase gene (*DHCR7*). *DHCR7* reduces 7-dehydrocholesterol (7-DHC) to cholesterol. Common clinical manifestations include dysmorphic facial features, mental retardation, and 2-3 toe syndactyly. Malformations of the brain including callosal abnormalities, Dandy-Walker variant, holoprosencephaly and prominent ventricles. We generated a hypomorphic *Dhcr7*^{T93M/} SLOS mouse model. Phenotypically *Dhcr7*^{T93M/} mice have mild dilatation of the third and lateral ventricles and 2-3 toe syndactyly. Ventricular dilatation was found in 54% of the mice by histological studies at 5 months of age (n=13). This malformation was not observed in newborns or at 2 months of age. We are using magnetic resonance imaging to serially study the development of ventricular enlargement in this SLOS mouse model. An initial study was performed comparing four control and four mutant nine-month-old males. Tissue and ventricular volumes were determined from 3-D reconstructions of fast spine echo images. No significant differences were observed for total brain tissue volume between control (297.26 mm³) and mutant (286.19 mm³) animals. This suggests that ventricular dilatation is not secondary to CNS tissues loss. Two mutant mice showed enlargement of the lateral ventricles (1.9 mm³ and 1.3 mm³) compared to control values (LV=0.470.19 mm³). Future experiments will focus on describing the time course of the development of ventricular enlargement by determining if sterol differences underlie penetrance of this malformation, if water diffusion and T₂ maps can detect parenchymal differences and whether simvastatin treatment can prevent the development of the ventricular enlargement.

Patients with XY Sex Reversal carry mutated SRY with Defective Calmodulin Recognition and Nuclear Import.

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SRY plays a key role in human sex determination as mutations cause male to female sex reversal as a consequence of complete gonadal dysgenesis. While many SRY mutations affect DNA binding and DNA bending activity it is not clear how others contribute to disease. Four XY females showed reduced SRY nuclear import. In two cases (R133W, R62G), their mutated SRY bound weakly to importin . In two other cases (R75N, R76P), SRY nuclear import was reduced but importin beta binding was normal (Harley et al., 2003, PNAS). This suggests the existence of an importin independent nuclear import pathway. The high mobility group (HMG) domain of SRY has two independent nuclear localization signals (NLS). The N-terminal NLS (nNLS) of SRY and the SRY-related testis factor, SOX9 bind calmodulin (CaM) in vitro (Sim et al., 2003, JBC). Here, we show that a CaM antagonist calmidazolium chloride (CDZ) blocks CaM recognition of SRY both in vitro and in vivo, leading to reduced nuclear import of SRY in treated cells. Fluorescence studies indicate that the conformation of SRY-CaM complex is dramatically different in R75N and R76P mutants. While CDZ reduced nuclear entry of wildtype SRY and the R133W (importin defective) mutant SRY, CDZ had no effect upon the R75N and R76P mutants suggesting that CaM-mediated import is defective in R75N and R76P. Taken together these data suggest that SRY binds CaM during nuclear import and that altered binding leads to reduced SRY nuclear import and sex reversal. This is the second demonstration of a human disease of protein nuclear import, the first being SRY-importin defects, and predicts that defective CaM binding could underlie diseases involving the approximately 100 other HMG box proteins encoded in the human genome.

Clinical outcome of patients who defer hematopoietic stem cell transplantation in favor of enzyme replacement therapy. *C.P. Lorentz¹, C.B. Whitley², S. Grewal², L. Charnas², C. Peters², E.G. Shapiro².* 1) Fairview University Medical Center, Minneapolis, MN; 2) Department of Pediatrics, University of Minnesota, Minneapolis, MN.

Hematopoietic stem cell (HSC) transplantation has become the standard of care for children with the most severe form of mucopolysaccharidosis (MPS) type I, i.e. Hurler syndrome. With the advent of enzyme replacement therapy (ERT), some parents make the informed decision to select low-risk laronidase (Aldurazyme) therapy in lieu of higher-risk HSC transplantation. While ERT may result in longer survival, failure of enzyme to cross the blood-brain barrier will result in progressive neurodegeneration with a deteriorating quality of life. Importantly, the rate of decline in severe MPS I patients without treatment is estimated to be about 20 IQ points per year (1.6 points per month). For example, we saw a 23-month-old female with characteristic facial features, dysostosis multiplex, and elevated urinary glycosaminoglycans. Subsequent mutation analysis found the patient to be homozygous for the Q70X mutation, thus, confirming the classic, severe MPS I phenotype. Although initial psychometric assessment was within normal limits, such patients inevitably decline in cognitive function. After extensive consultation regarding treatment options, emphasizing the distinctions in neurologic efficacy, the parents elected to initiate weekly ERT rather than HSC transplantation. Over the course of 14 months on treatment, the parents appreciated increased limb mobility and some developmental progress. They declined reconsideration of HSC transplantation. Serial evaluations will determine the neurologic outcome, quality of life, and longevity for such individuals. Immediate benefits of ERT may distract parents from considering the inevitable, long-term neurodegeneration that a child will experience. When counseling parents about combined ERT and HSC therapy, this neurodegeneration must be emphasized.

X-linked dominant Conradi-Hünemann syndrome in a male patient due to single gene mosaicism. H.R.

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Conradi-Hünemann syndrome, also known as X-linked dominant chondrodysplasia punctata 2 (CDPX2; MIM 302960), is characterized by a bilateral, asymmetric expression of various skeletal and skin abnormalities, including chondrodysplasia punctata (epiphysic stippling), shortening of long bones and ichthyosis. The disorder is caused by a deficiency of the sterol⁸⁻⁷ isomerase due to mutations in the *EBP* gene on chromosome Xp11.22-23. As a consequence of this enzyme deficiency, patients have elevated plasma levels of cholesta-8(9)-en-3-ol but usually normal levels of cholesterol. Since the disorder is recognized almost exclusively in females it has been assumed to be lethal in males. We here report a 24-old male patient initially diagnosed with CDPX2 on the basis of his clinical presentation. The patient is severely retarded, both mentally and developmentally, and has a variety of skeletal (polydactyly, dwarfism, scoliosis, unilateral rhizomelic shortening) and skin abnormalities. Sterol analysis in plasma and cultured skin fibroblasts revealed elevated levels of cholesta-8(9)-en-3-ol. Surprisingly, mutation analysis of the *EBP* gene revealed apparent heterozygosity for a 429delG mutation although the patients karyotype was 46,XY. The different ratios of mutated versus wild-type allele in lymphocyte and fibroblast DNA indicate that the patient is mosaic for a single mutation in the *EBP* gene.

GLUTAMATE CYSTEINE LIGASE CATALYTIC SUBUNIT TRINUCLEOTIDE REPEAT

POLYMORPHISM AND TYPE I DIABETES AGE-AT-ONSET. L. Bekris¹, C. Shephard¹, J. Graham², B.

McNeney², J. Shin², M. Zarghami³, F. Farin¹, T. Kavanagh¹, A. Lernmark³. 1) Environmental Health, University of Washington, Seattle, WA; 2) Statistics and Actuarial Science, Simon Fraser University, Burnaby, B.C; 3) Department of Medicine, University of Washington, Seattle, WA.

Glutathione (GSH) protects against reactive oxygen species (ROS)-mediated cell injury. Glutamate cysteine ligase (GCL), the rate limiting enzyme in the synthesis of GSH consists of catalytic (GCLC) and modifier (GCLM) subunits. Type 1 diabetes (T1D) is an autoimmune disease involving auto-reactive T-cells and ROS, which may cause pancreatic beta-cell destruction. We hypothesized that GCLC may be a susceptibility factor for T1D. A polymorphic GAG trinucleotide repeat (TNR) exists in the 5'untranslated region of the GCLC gene. The aim of this investigation was to determine the frequency of this polymorphism and associations with age-at-onset and islet autoantibodies in a group of new onset 0-34 year old T1D (n=186) and in controls (n=189) from Sweden. Results show: 1) In the T1D, there is marginal statistical evidence for a higher proportion of females when the GCLC TNR 8 allele is present (p-value: unadjusted 0.021, Bonferroni-corrected 0.063) and 2) there is marginal statistical evidence for a higher frequency of the GCLC TNR allele 8 (unadjusted p-value, 0.019, Bonferroni-corrected p-value, 0.057) and for a higher frequency of the GCLC TNR genotype 7/8 (unadjusted p-value, 0.013, Bonferroni-corrected p-value, 0.078) in the younger 0-14 year old T1D than in the 15-34 year old T1D. This study suggests that oxidative stress, due to the GCLC TNR polymorphism may precede type 1 diabetes onset. The GCLC TNR 8 allele and the GCLC TNR 7/8 genotype may be associated with a younger age-at-onset in T1D. Since GCLC TNR 7 and TNR 8 have been reported to be associated with lower GSH levels than GCLC TNR 9 and GSH has been reported to be depleted in T1D we speculate that the GCLC TNR polymorphisms may contribute to T1D risk.

Early epileptic encephalopathy and neonatal diabetes, associated with mutation in ATP-sensitive potassium channel, Kir6.2. *N. Nahi-Buisson¹, C. Bellanne-Chantelot², M. Eisermann³, R. Nabbout¹, N. Bach⁵, S. Nivot⁵, P. Plouin², JJ. Robert⁴, P. de Lonlay¹.* 1) Neuropediatrics, Necker Enfants Malades Hospital, Paris; 2) Molecular Biology Necker Enfants Malades Hospital, Paris; 3) Neurophysiology, Necker Enfants Malades Hospital, Paris; 4) Pediatric Endocrinology Necker Enfants Malades Hospital, Paris; 5) Pediatric Unit, Caen.

Heterozygous activating mutation in the gene encoding the Kir6.2 subunit of the ATP-sensitive potassium (KATP) channel have recently been described as a cause of neonatal diabetes occasionally associated with heterogeneous neurological disorders. We present here a female who presented from birth, feeding problem, developmental delay, and mild dysmorphic features. Intrauterine growth retardation was observed at 28 weeks of gestation. At 3 months of age, she developed neonatal diabetes and a severe epileptic encephalopathy, characterized by epileptic spasms, tonic seizures, and an hypsarhythmic pattern on EEG. Brain MRI, Spectro MRS, and metabolic investigations were normal. Mutation analysis of the Kir6.2 gene (KCNJ11) revealed a C166F missense de novo mutation. Diabetes was controlled by continuous doses of Insulin. Based on in vitro studies, a trial of sulfonylureas is performing, as the antiepileptic drugs were inefficient on the epileptic encephalopathy and the child required high doses of insulin with corticotherapy. The mechanism of this epileptic encephalopathy is still elusive. We discuss several hypotheses, such as the direct role of the activation of the (KATP) channel, a channelopathy with a secondary disturbed excitability of the cortex, or the role of the intrauterine growth retardation in the genesis of this severe epileptic encephalopathy. In conclusion, activating mutation Kir6.2 should be regarded as a possible cause of West syndrome when associated with diabetes. A treatment by sulfonylurea is questionable.

Investigation of Simvastatin Treatment in Smith-Lemli-Opitz Syndrome. C.A. Wassif, L.S. Correa-Cerro, F.D. Porter. NICHD/HDB/NIH, Bethesda, MD.

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive, malformation syndrome due to mutations of the 7-dehydrocholesterol reductase gene (DHCR7). DHCR7 converts 7-dehydrocholesterol (7DHC) to cholesterol. As a result of this perturbation, SLOS patients have increased 7DHC levels. Dietary cholesterol replacement is the standard current treatment. Recently attempts have been made to treat SLOS patients with simvastatin in order to reduce the levels of 7DHC both in the periphery and the central nervous system. One study of simvastatin therapy in SLOS patients showed a decrease in 7DHC and an increase in cholesterol. To study the efficacy of simvastatin treatment in SLOS, we have undertaken a multifaceted approach. First, by studying the effects of simvastatin in cell culture, and second, by generating a hypomorphic, viable SLOS mouse model. This was done by "knocking in" a T93M mutation. Mice homozygous for a null disruption (D) of *Dhcr7* die soon after birth. T93M mice were phenotypically normal; however, T93M/D mice had 2-3 toe syndactyly. Sterol profiles of liver, cortex, midbrain, and kidney in both one day old and six weeks old mice showed elevated 7DHC. 7DHC levels were higher in the T93M/D compared to T93M/T93M mice. Treatment of T93M/D mice with simvastatin (10mg/kg/d) for three weeks significantly decreased 7-DHC levels in some peripheral tissues, and decreased 7DHC levels in the cortex. In culture, we have tested 51 SLOS cell lines for their ability to produce cholesterol, and have found a correlation between fractional cholesterol synthesis and severity. SLOS cell lines that have no residual cholesterol synthesis die when treated with simvastatin. Conversely, when cell lines that retain some residual cholesterol synthesis capabilities are treated, 7DHC levels decrease and fractional cholesterol synthesis is increased. Treatment of cells that have been allowed to accumulate 7DHC and then treated with simvastatin show a reduction in 7DHC and a rise in cholesterol. The reduction in 7DHC and the rise in cholesterol levels corresponds to an increase in the levels of the *Dhcr7* transcript. We are continuing to investigate whether increased DHCR7 transcription is mediated by the SREBP pathway or some other mechanism.

Two severe congenital hyperinsulinism resulting from a genome-wide paternal isodisomy and a paternal trisomy combined with a loss of maternal allele. *I. Jiurgea¹, D. Sanlaville¹, J.C. Fournet¹, C. Sempoux², C. C. Bellanné⁴, F. Jaubert¹, F. Brunelle¹, J. Rahier², V. Geromet³, J.M. Saudubray¹, C. Nihoul-Fékété¹, J.J. Robert¹, M. Dunne⁵, J. Feingold¹, M. Vekemans¹, C. Junien¹, C. Gicquel⁶, P. de Lonlay¹.* 1) INSERM U393, Pediatric depart, Hosp Necker-Enfants Malades, paris, France; 2) Department of Pathology, Universit de Louvain, Brussels; Pasteur-Cerba Laboratory; 3) Pasteur-Cerba Laboratory, 95066 Cergy Pontoise Cedex 9; 4) Department of Biology, Hpital Saint-Antoine, Paris, France; 5) Division of Physiology and Pharmacology, School of Biological Science, The University of Manchester, Oxford Road, Manchester; 6) Department of Cytogenetics, Hpital Trousseau, Paris, France.

Focal hyperinsulinism (HI) is caused by a limited somatic event, the loss of maternal alleles in the 11p15 region, responsible for the reduction to homozygosity of a paternally inherited mutation on the ABCC8 or the KCNJ11 gene, coding for a potassium channel. HI can also be observed in Beckwith-Wiedemann syndrome (BWS). We report here two patients with severe HI requiring partial pancreatectomy. Patient 1 had a BWS and patient 2 had a suspected focal HI. In both cases, histology showed a focal unusual aspect of the endocrine tissue. A loss of the maternal allele on all 23 chromosomes was found in the pancreatic lesion and in some leukocytes from patient 1. Paternal isodisomy in leukocytes was confirmed by a 17p13.3 southern blot and rhesus analysis. A paternal trisomy on half of the chromosomes with two genetically different paternal alleles were found in pancreatic lesion of Patient 2. In the other half a loss of maternal allele was observed. In both patients, potassium channels were functional on hyperplastic pancreas and no mutation of ABCC8 and KCNJ11 genes was detected. We want to discuss the role and the mechanism of these rearrangements. In particular they could reduce recessive mutations to homozygosity and/or deregulate the pancreatic expression of imprinted genes located in or outside the 11p15 region, leading to abnormal pancreatic histology and severe hypoglycemia.

Mesenteric mass associated with type III Gaucher disease. *M. Dudek*¹, *W.J. Rhead*², *B. Camitta*², *D. Margolis*², *M. Hernanz-Schulman*³, *B.A. Maxfield*⁴, *G.E. Tiller*¹. 1) Div Med Genetics, Dept Pediatrics, Vanderbilt Univ Sch Med, Nashville, TN; 2) Dept Pediatrics, Med College of Wisconsin, Milwaukee, WI; 3) Dept Radiology, Vanderbilt Univ Sch Med, Nashville, TN; 4) Dept Radiology, Med College of Wisconsin, Milwaukee, WI.

Gaucher disease (GD) is an autosomal recessive lysosomal storage disorder caused by mutations in the gene for beta-glucocerebrosidase. We report an 11 year old Mexican patient with GD type III who developed a mesenteric mass secondary to her disease. She was diagnosed at age 2 years when she presented with organomegaly. She is homozygous for the L444P mutation, and has been receiving enzyme replacement therapy consisting of biweekly infusions of Ceredase or Cerezyme since the time of diagnosis. Chronic problems have included mild developmental delay, growth retardation, abdominal distention, and constipation. At 11 years of age, she presented with severe abdominal pain and no bowel movement for one week. A CT scan revealed a large, multicystic, anterior abdominal mass measuring 11x14cm. At laparotomy, cystic portions were decompressed, but the mass could not be excised due to proximity to the mesenteric vasculature. Histologic analysis of cystic fluid and multiple soft tissue nodes demonstrated Gaucher cells, but failed to identify any neoplastic cells. The patient has been managed postoperatively for four months with stool softeners and weekly infusions of 90u Cerezyme/kg, with decrease in volume of the mass and no progression of symptoms. A similar case has been reported previously (*Ped Radiol* 32:674, 2002). We recommend that patients with GD and chronic gastrointestinal symptoms warrant periodic evaluation, including appropriate imaging studies when indicated.

CNS involvement in Fabrys disease: an image study during 24 months of ERT. *L.B. Jardim^{1,2}, L. Vedolin⁴, C. Cecchin², L. Kalakun², F. Aesse⁴, C. Pitta-Pinheiro⁴, J. Marconato⁴, R. Giugliani^{2,3}.* 1) Department of Internal Medicine, UFRGS, Brazil; 2) Medical Genetics Service, Hospital de Clinicas de Porto Alegre, Brazil; 3) Department of Genetics, UFRGS, Brazil; 4) Department of Neuroradiology, Hospital Moinhos de Vento.

Objectives: To access the neuronal involvement in Fabrys disease (FD) with MRI, MRA and proton MR spectroscopy, during 24 months of ERT. **Methods:** A brain MRI and MRA study of 7 male patients with FD, mean age: 31 y, were performed just before their inclusion in a compassionate use trial with agalsidase-alpha. A standard neurological examination generated a score which varied between zero (normal examination) and 85. Presence and location of gray and white matter (WM) lesions, the presence of vascular occlusion or ectasia at MRA were determined, as well as their relation with age, symptoms and neurological examination. **Results:** (a) Baseline: There were few, previous CNS signs and symptoms. NE CNS scores varied from zero to 3. Three patients showed a widespread pattern of deep WM lesions, mainly in frontal and parietal lobes. (b) Evolution of NE scores in 24 months of ERT: NE CNS scores were all similar, during the period under study. However, two patients presented NE scores significantly different from the others on the 24th month: case 1 had a worse CNS score than others, and case 6, whose NE was completely normal (CI of 95%). (c) Evolution of MRI during 24 months of ERT: At the 12th month, MRI worsened in 2/7 and was stable in 5/7 patients. Patients with progression of WM lesions, mainly in frontal lobes, cases 1 and 2, were related and the eldest of the sample. At the end of 24th month, the MRI of the remaining 5 patients was stable in 3/5, and worsened again in case 1. Finally, WM lesions disappeared in case 6. **Discussion:** MRI seemed to be a better parameter than neurological examination to follow the CNS evolution of FD patients. MRI also showed that, during ERT, WM lesions, although still silent, can progress in some patients, and disappear in others, and that this effect could be related to their ages at the inclusion on ERT.

Support: TKT.

Identification of mutations and short-term experience of enzyme replacement therapy in Korean patients with Fabry disease. *G-H. Kim¹, J-H. Choi², S-S. Kim³, Y. Kim³, H-W. Yoo^{1, 2, 3}.* 1) Med Genetics Clinic & Lab, Asan Medical Center, Seoul, Korea; 2) Department of Pediatrics, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; 3) Genome Research Center for Birth Defects & Genetic Disorders, Asan Medical Center, Seoul, Korea.

Fabry disease is an X-linked recessively inherited metabolic disorder, which results from the deficient activity of the lysosomal hydrolase alpha-galactosidase A (-Gal A) leading to the systemic pathologic accumulation of globotriaosylceramide (GL-3). Recent clinical trials have demonstrated that enzyme replacement therapy (ERT) in patients with Fabry disease is effective in both reducing the storage end-product GL-3 and alleviation of symptoms and signs in multi-organ systems. This study aims at characterization of molecular defects of Korean patients with Fabry disease and evaluating the efficacy of ERT during a short-term period. We have identified 10 different mutations of -Gal A in 15 patients from 10 unrelated Korean families with Fabry disease; M42V, E66Q, Q99fs121(c.297_298del), R112C, D231G, D266N, L268fs(c.803_806del), R342X, T412fs(c.1235_1236del), and Y866X. In order to evaluate the effects of these mutations on the -Gal A activity, we constructed E66Q, R112C, and D266N cDNA from a wild type -Gal A. COS-7 cells transfected with these mutant (R112C, D266N) cDNA showed complete loss of its enzyme activities (about 4 and 2 % of wild type-Gal A, respectively), except E66Q with a residual activity (about 30%). Nine male patients and one symptomatic female heterozygote have been on ERT with agalsidase beta (Fabrazyme) at the standard dose of 1 mg/kg every 2 weeks for ranging from 6 months to one year. All patients have shown remarkable reduction of plasma GL-3 level within 3 months after ERT. It has been normalized within 6 months in most cases. However, urinary GL-3 excretion has persisted without significant reduction within 3-6 months after ERT. Renal function remained normal based on serum creatinine levels and creatinine clearance rate. There was no adverse event except infusion-associated fever and chillness. Also, other clinical profiles were monitored during ERT to verify its efficacy.

Haplotype diversity and genetic determinants of the therapeutic response. *R. Sanchez, H. Vasquez, D. Labuda, V. Yotova, F. Costea, E. Levy, D. Sinnott.* Centre de recherche Hopital Ste-Justine, Montreal University, Montreal (Quebec), Canada, H3T 1C5.

The inflammatory bowel disease (IBD) is a complex genetic disorder characterised by at least two major disorders: Crohns disease (CD) and ulcerative colitis (UC). IBD is the most common intestinal disease in Canadian children, with approximately 25% of all cases diagnosed before the age of 20. Different therapeutic approaches are used, 1) combinations of glucocorticosteroids and amino-salicylic acid 2) immunosuppressors drugs (for refractory and steroid-dependent cases) and recently 3) biological drugs such as monoclonal anti-TNF- and IL-10. Genetic factors influence the individuals response to treatment and susceptibility to drug toxicity. Determination of the underlying genetic polymorphisms may be of clinical value in predicting adverse or inadequate response to certain therapeutic agents. In this study we identified genetic variation in candidate genes in order to be able to perform pharmacogenetics investigations in respect to IBD therapeutic responses and toxicity. The candidates genes (TNF-, IL-10, NFB, IKB-, IKAP and MDR-1, MRP-1) are implicated in cellular and molecular pathways involved in the metabolism of commonly administered drugs in IBD patients. Genotypes of childhood cases (n=334) and worldwide population controls were determined by PCR-allele specific oligonucleotide (ASO) hybridization assays. These data were used to determine the haplotype structures and frequencies. The resulting haplotypes are being used for association and/or pharmacogenetic studies. This study will help to increase our understanding of the genetic factors that modify IBD therapeutic response. Supported by VRQ (Valorisation Recherche Qubec).

AAV vector mediated co-expression of FGE with ASA for gene therapy of metachromatic leukodystrophy. T.

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Metachromatic leukodystrophy (MLD) is a lysosomal storage disorder caused by a deficiency of arylsulfatase A (ASA). Accumulation of sulfatide results in a variety of neurologic symptoms. No effective therapy is available at this moment. Gene therapy may be an important option for treatment of MLD. It was reported that posttranslational conversion of a cysteine residue to formylglycine is essential for ASA activity. Accordingly, co-expression of formylglycine generating enzyme (FGE) encoded by the sulfatase modifying factor 1 (SMUF1) gene with ASA should be important for mass production of active ASA. In this work, we studied the utility of FGE in gene therapy for MLD. When FGE expression plasmid was cotransfected with ASA expression plasmid into 293, HeLa, and Cos cells, a striking increase of ASA activity was observed. These plasmids were injected into liver of MLD knockout mice using the hydrodynamics-based technique. A synergistic effect of FGE on ASA was also confirmed in both liver and serum *in vivo*. We now generated AAV vectors expressing ASA and FGE. The AAV vectors were pseudotyped with type 1, 2 or 5 capsid and purified by a novel procedure based on the density-gradient centrifugation using iodixanol. The final preparations showed extremely high transduction efficiency both *in vitro* and *in vivo*. Two to three folds enhancement of ASA activity by co-expression of FGE was observed in HeLa cells transduced with AAV vectors. These results indicate that co-expression of FGE with ASA is important for MLD gene therapy. AAV vectors expressing ASA and FGE are useful for further evaluation of gene therapy approaches for MLD.

An Ethnic Specific Polymorphism in Glutamate Cysteine LIgase May Affect Enzyme Activity. *A.S. Willis^{1,2}, T.M. Le², M.L. Freeman³, K.R. Sekhar³, M.S.B. Kennedy¹, S. Dawling⁴, L.J. Roberts⁵, M.L. Summar^{1,2}.* 1) Center for Human Genetics Research, Vanderbilt Univ, Nashville, TN; 2) Dept Pediatrics, Vanderbilt Univ., Nashville, TN; 3) Dept Radiation Biology, Vanderbilt Univ., Nashville, TN; 4) Dept Pathology, Vanerbilt Univ., Nashville, TN; 5) Dept Pharmacology, Vanderbilt Univ., Nashville, TN.

Glutamate cysteine ligase (GCL) catalyzes the first, rate-limiting step in the de novo synthesis of glutathione, an important tripeptide. One of the most important functions of glutathione is the scavenging of free radicals to prevent oxidative damage, which is thought to play a role in many diseases, such as neurodegeneration and cancer. We have previously identified a nonsynonymous polymorphism C1384T in GCLC. The rare allele of this polymorphism changes a conserved proline to serine at position 462 and has only been observed in individuals of African descent. We have undertaken the study of the functional aspects of this polymorphism. Using clones of the coding region of both human GCLC and GCLM, we have expressed both versions of the GCLC C1384T polymorphism in *E. coli*. Western blot demonstrates expression of soluble normal sized GCLC protein. We have optimized an HPLC method for detection and quantitation of -glutamylcysteine, the primary product of GCL as an improvement over our previous method, which monitored production of inorganic phosphate as a result of ATP usage. Using the HPLC method for activity assays, lysates containing human GCL bearing the C allele had increased levels of -glutamylcysteine over lysates in which no human GCL was expressed. However, -glutamylcysteine levels in lysates with GCL bearing the T allele were not increased over background control, suggesting that this version of GCL had little to no activity in this system. We are continuing to explore the effect of the C1384T polymorphism on GCL activity and feel that these findings may be important in a number of diseases where oxidative stress plays a role.

The molecular basis of Gaucher disease in South Africa. *S. Arndt, M. Ramsay.* Division of Human Genetics, National Health Laboratory Service and School of Pathology, University of the Witwatersrand, Johannesburg, South Africa.

Gaucher disease (GD) is a recessively inherited lysosomal storage disorder. The gene responsible for GD is located on chromosome 1q21 and has a highly homologous pseudogene in close proximity resulting in a number of complex mutations due to unequal recombination events. GD occurs at a particularly high frequency in Jewish people of Eastern European ancestry. They share a well-characterized mutation spectrum in the GBA gene, with a large number of point mutations. All these mutations have also been observed in other Caucasian populations around the world, including South Africa. In contrast, very few of these mutations were found in the South African Black GD patients. Seventeen unrelated Black GD patients were studied and 33/34 disease causing mutations were identified. A 3 bp deletion mutation in exon 3 (delta T36) was found in 12/34 (0.353) alleles. This mutation has only been observed in Black patients. In addition, 6/34 (0.177) alleles were identified as the recombinant allele recNciI. Interestingly, 5/17 (0.294) Black patients were compound heterozygotes for the same two mutations. The remaining 13/34 disease causing mutations were missense and nonsense mutations of which seven are novel. Our results have illustrated a different mutation spectrum for GD in South African Black patients, and this information will be used to improve our molecular diagnostic service. Haplotype studies are in progress and preliminary results for two markers (D1S2777 and D1S2140) show complete LD with the frequently observed delta T36 mutation, supporting a founder hypothesis for this mutation. Ethics approval for this research project has been obtained (M030201).

An algorithm for rapid mutation identification in peroxisome biogenesis disorder patients in the Zellweger syndrome spectrum. *S. Steinberg*^{1,2}, *G. Cutting*³, *L. Wei*¹, *L. Chen*³, *A. Moser*^{1,2}, *H. Moser*^{1,2}, *N. Braverman*³. 1) Dept Neurogenetics, Kennedy Krieger Inst, Baltimore, MD; 2) Dept Neurology, Johns Hopkins University School of Medicine, Baltimore, MD; 3) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD.

Zellweger syndrome (ZS), neonatal adrenoleukodystrophy and infantile Refsum disease comprise the Peroxisome Biogenesis disorder-ZS spectrum (PBD-ZSS). These are severe, multisystemic developmental disorders caused by a failure to assemble peroxisomes. Measurement of plasma very long chain fatty acid levels is the hallmark used for biochemical diagnosis, but a variety of other tests are available. Molecular diagnosis provides a number of tangible benefits over biochemical diagnosis, but the heterogeneity of PBD-ZSS makes mutation identification challenging. These are autosomal recessive disorders caused by defects in 12 different PEX genes. However, the majority of patients (>90%) have defects in one of 5 genes: *PEX1*, *PEX6*, *PEX26*, *PEX12* & *PEX10*. Thus, we devised an algorithm for the systematic molecular analysis of PBD-ZSS patients. Genomic DNA was isolated from cultured fibroblasts from 91 PBD-ZSS not previously studied by complementation analysis. A maximum of 13 PCR amplicons were used for sequence analysis of selected exons from the 5 most common genes. Pathological mutations were identified in 67 (74%) of patients. Overall, 23 novel mutations were identified. This systematic, hierarchical approach to mutation identification provides a molecular diagnosis in about three-quarters of patients and thus is a valuable tool for the diagnostic laboratory.

Defective fuel metabolism in the *Abcd3*^{-/-} mouse is related to inappropriate activation of PPAR. *I. Silva-Zolezzi*^{1,2,3}, *S. Bradley*¹, *D. Valle*¹, *G. Jimenez-Sanchez*^{1,3}. 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) PhD. program in Molecular Biomedicine, CINVESTAV, Mexico; 3) National Institute of Genomic Medicine, Mexico.

There are four known human half ABC transporters in the peroxisomal membrane: ALD, ALDR, PMP70 and P70R, encoded by *ABCD1*, 2, 3, and 4, respectively. Mutations in *ABCD1* cause X-linked adrenoleukodystrophy, function and disease associations of the other three transporters are unknown. *Abcd3*^{-/-} mice are viable; have reduced hepatic glycogen; medium chain dicarboxylic aciduria; defective phytanic and pristanic oxidation; and defective non-shivering thermogenesis. We postulated that PMP70 contributes to the peroxisomal transporter of branched fatty acids. In the absence of PMP70, phytanic and pristanic acids accumulate, leading to inappropriate activation of PPAR, in turn activating fasting fuel homeostasis (FFH). This hypothesis is supported by our demonstration of increased hepatic expression of PPAR targets in *Abcd3*^{-/-} mice. To further analyze the molecular mechanisms underlying this phenotype, we performed an expression analysis in *Abcd3*^{-/-} livers and controls with Affymetrix U74Av2 microarrays. We found expression changes (EC) in at least 50 genes PPAR regulated genes, including those we previously observed by Northern blot. In total, we found EC in 209 genes including 93 with an increased and 116 with a decreased EC over controls. We classified the genes by biological process according to Gene Ontology: 40 (19%) were related to lipid metabolism, including genes related to fatty acids anabolism and catabolism, formed the largest group, most showing an increased EC; 28 (13%) were related to the immune response (IR), most (22) with a decreased EC consistent with the known anti-inflammatory role of PPAR. To evaluate the physiological relevance of the altered expression of IR-related genes, we are currently testing inflammation on *Abcd3*^{-/-} mice. These results support our hypothesis that the severe metabolic phenotype of *Abcd3*^{-/-} mice is related to inappropriate PPAR activation leading to FFH activation and emphasize the profound and widespread consequences that can occur in a monogenic defect.

Correlations of genotype-phenotype in Fabry disease. *L.W. Lai, Y.H. Lien.* Department of Medicine, University of Arizona, Tucson, AZ.

Fabry disease is an X-linked recessive disease caused by mutations in the α -galactosidase A gene. Molecular diagnosis of Fabry disease is important for genotype-phenotype correlations, early diagnosis, and detection of carrier status. Genomic PCR and RT-PCR sequencing of α -galactosidase A were performed in patients from 16 unrelated Fabry families. Five novel mutations (R301G, Y222X, T410A, M42L, and L286P) and 10 previously reported mutations (P40S, S65T, R112C, R112H, N215S, R301Q, R220X, R227X, 777delA, and 1188delC) were identified. One mutation previously diagnosed as a missense mutation S65T, was found to be a splice-site mutation (IVS1-1GC) resulting in an insertion of 14 bp and a frameshift stop at codon 106. For 30 individuals at risk, Fabry disease was diagnosed in 5 males and carrier status in 12 females. Structure-functional analysis of missense mutations predicted that P40S affects active site; N215 affects glycosylation site; T410A may affect phosphorylation site; and M42L, R112C, R112H, R301G, R301Q and L286P cause misfolding. Genotype-phenotype correlations are: M42L, N21S, R301G and R301Q correlate with atypical variant of Fabry disease, while the rest correlate with classic Fabry disease. The M42L mutation is associated with renal variant as the index male patient developed ESRD at age of 70 without other Fabry stigmata. Other unique clinical manifestations were found in two males with severe lymphedema (R227X and 777delA), one male with transient complete heart block at age of 80 (R301Q), one female with FSGS and renal failure in spite of a benign missense mutation N215S, and one female with RPGN responsive to cytotoxic therapy (R301Q). Our findings indicate that molecular diagnosis of Fabry disease has important clinical values in identifying Fabry patients and carriers, and predicting prognosis based on genotype-phenotype correlations. Sequencing both genomic DNA and mRNA provides complete molecular information and avoids genotype misdiagnosis. Furthermore, chemical chaperons are currently developed as a potential therapy specific for patients with missense mutations, which cause protein misfolding. Molecular diagnosis will be useful for identifying such patients who may benefit from chaperon therapy.

Infantile and Juvenile-onset Glycogen Storage Disease type II in a Druze Kindred from Northern Israel:

Genotype Phenotype Correlation. *H. Mandel*¹, *C. Shochat*², *S. Korem*², *L. Sanas*², *O. Mesika*¹, *R. Gershoni-Baruch*¹, *D. Bercovich*². 1) Metabolic unit, Department of Genetics, Rambam Hospital, Technion Faculty Medicine, Haifa, Israel; 2) Department Human Molecular-Genetics, Pharmacogenetics, Migal-Galilee, Biotechnology Center, Kiryat-Shmona, Israel.

Objective: To characterize the phenotypes of patients with glycogen storage disease type II (GSD II) in a Druze kindred from northern Israel and correlate them with genetic defects. **Background:** GSD II is an autosomal recessive disease caused by deficiency of lysosomal acid alpha-glucosidase (GAA). It occurs in different clinical forms and manifests biochemical and genetic heterogeneity. **Methods:** We performed clinical, biochemical and genetic study including 17 patients, from 5 families in one kindred. DNA samples were collected from members of 4 generations including 5 patients, 10 obligatory heterozygotes, and 50 family members. We screened the 20 exons of the GAA gene using denaturing high performance liquid chromatography (DHPLC) and sequenced fragments with different chromatograms. **Results:** Three phenotypes were identified: *Type 1*. Infantile, early-onset, rapidly progressive cardiomyopathy, skeletal myopathy, and death prior to age 1y; *Type 2*: Similar to type 1, but prolonged survival till age 3y; *Type 3*: Juvenile-onset, after age one year, slowly progressive skeletal myopathy without cardiac symptoms. Two missense mutations were identified: a novel A1651G (D404N) at exon-8, and a previously described T1505C (L355P) mutation in exon 6 (reported as associated with infantile type). Three patients whose parents are carriers of D404N mutation had phenotype type 1. Of 3 patients, homozygotes for the L355P, two had type 2, and one type 3. Eleven patients who were compound heterozygotes of these two mutations presented all three phenotypes. **Conclusions:** 1) In this kindred no clear correlation between the mutation and phenotype of GSD II was found. 2) A reliable carrier detection can be offered in this kindred. In a community where termination of pregnancy due to severe genetic disease is often not acceptable, a reliable carrier detection before first cousin marriage is planned, could be an acceptable option.

The relationship between genotype and phenotype in Fabry disease. *A. Mehta*¹, *E. Schäfer*², *M. Beck*³, *U. Widmer*⁴, *G. Sunder-Plassmann*⁵, *A. Gal*². 1) Department of Haematology, Royal Free Hospital, London, United Kingdom; 2) Institute of Human Genetics, University of Hamburg, Hamburg, Germany; 3) Department of Paediatrics, University of Mainz, Mainz, Germany; 4) Department of Medicine, University of Zurich, Zurich, Switzerland; 5) Department of Medicine III, Medical University, Vienna, Austria.

Fabry disease is an X-linked disorder caused by a deficiency of the enzyme α -galactosidase A. Accumulation of the enzyme substrate globotriaosylceramide in lysosomes leads to organ failure and premature death. Males with the classic form of the disease typically have undetectable enzyme activity and multi-organ involvement. Some males, however, are less severely affected. More than 250 different mutations in the gene encoding α -galactosidase A (*GAL A*) have been identified in patients with Fabry disease. It has been suggested that mutations leading to complete loss of enzyme function, e.g. by virtue of a premature stop codon, are associated with classic Fabry disease, whereas mutations resulting in amino acid substitutions result in less severe disease. We report an analysis of the relationship between genotype and phenotype in patients in FOS the Fabry Outcome Survey. α -Galactosidase A was found to be concordant in 27 out of 32 families with two or more affected males, suggesting a limited correlation between genotype and enzyme activity. Consistent with this finding, when clinical symptoms, enzyme activity and localization of missense mutations were considered together, a limited correlation was observed between phenotype and genotype. Future analysis of the functional consequences of *GAL A* mutations at the molecular level may help to elucidate further the genotypephenotype relationship in Fabry disease.

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Genetic susceptibility to the toxic effects of thimerosal in inbred mouse strains. *I.K. Mehta, D.W. Kim, J. Gitschier.*
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Thimerosal, a commonly used preservative that is approximately 50% ethylmercury by weight, has been implicated as a potential cause of autism. We hypothesize that genetic susceptibility to mercury toxicity could exacerbate the neurotoxic effects of mercury and potentially underlie the development of autism and/or neurodevelopmental disorders in some cases. To assess the genetic influences in an animal model, we are investigating the effects of thimerosal in a panel of inbred mouse strains (C57BL/6, 129S1/SvIm, BALB/cBy, C3H/He, DBA/2, and FVB/N, NZW, and AJ). To mimic the pediatric timetable of childhood vaccination, we subcutaneously injected mice with thimerosal on postnatal day 7. Of the mouse strains tested, most show susceptibility to the toxic effects of thimerosal, exhibiting death within 1 month post-injection with thimerosal. In contrast, one strain, namely 1291/SvIm, shows significant survival, at least 6 months post-injection. To evaluate the metabolic fate of thimerosal in thimerosal-susceptible vs. thimerosal-resistant strains, thimerosal concentrations were measured in brain, liver, kidney and heart derived from C57BL/6 and 129SI/SvIm mice. In addition, histological analysis of thimerosal injected vs. mock-injected C57BL/6 and 129SI/SvIm mice are being conducted. Preliminary data suggest that these two strains may metabolize thimerosal differently, allowing for a differential survival at lethal doses. We are currently assessing the inheritance pattern for thimerosal resistance and ultimately plan to identify the genetic loci responsible for this trait.

Mutational Analysis of ATP7B and Genotype-Phenotype Correlation in Indian Wilson Disease patients. R.

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Wilson disease (WD) is an autosomal recessive disorder of copper transport. The gene ATP7B responsible for Wilson disease produces a protein, which is predicted to be a copper-binding P-type ATPase, homologous to Menkes disease gene ATP7A. This study was aimed to detect disease-causing mutations and to determine the possible genotype-phenotype correlation. We have analyzed 43 unrelated Indian Wilson disease families, including 43 patients. We have characterized 22 mutations and 6 polymorphisms by SSCP and direct sequencing of all 21 exons. Among them 21 were novel mutations and 5 were novel polymorphisms. Of these mutations, fourteen were insertion/deletion, five were missense, two were splice site and one was nonsense. The frequency of mutations in WD chromosomes were accounted to be 19% in exon 13, 9% in exon 15, 6% in exon 18, 3.4% in exon 8, 12 and 16, 2.3% in exon 4 and 1.2% in exon 7, 9, 11 and 21. The mutations Pro992His, Ile1102Thr, 2997insA, 3031insC were observed in 24% of the WD chromosomes. Four homozygous patients, two for 2977insA, two for 3031insC mutations and twelve heterozygous patients, six for Ile1102Thr, two for Lys910-ter, two for Ala1002Thr and two 3424insC showed entirely different phenotypes. All WD patients demonstrated significantly decreased copper-stimulated ATPase activity, which was fraternized with elevated levels of liver biopsy copper for individual mutation. The identified mutations provided for at least part of the wide phenotypic variation observed in Wilson disease. The mutations were found in the compound heterozygous state together with other mutation and homozygosity was rarely observed.

The prevalence of premature termination codons in Carbamyl Phosphate Synthetase I Deficiency. *M. Summar*^{1,2}, *A. Eeds*², *L. Hall*¹, *M. Yadav*¹, *B. Christman*³, *F. Barr*¹, *M. Ryan*¹, *A. Putnam*¹, *S. Summar*¹, *M. Arvin*¹, *Urea Cycle Disorders Consortium*. 1) Dept Pediatrics/Div Genetics, Vanderbilt Univ Medical Ctr, Nashville, TN; 2) Center for Human Genetic Research, Dept Molec Physiology, Vanderbilt Univ, Nashville, TN; 3) Dept Pulmonary Medicine, Vanderbilt Univ, Nashville, TN.

The rate-determining enzyme in the urea cycle is carbamyl phosphate synthetase I (CPSI). CPSI Deficiency (CPSID), characterized by hyperammonemia, is autosomal recessive. Efforts to genotype patients by sequencing gDNA and cDNA have not always resulted in the detection of both alleles at the RNA level, though two mutations are almost always found when sequencing patient gDNA. We hypothesize that alleles not represented in cDNA constructs contain a mutation that results in the instability of that RNA message due to splicing defects and frameshifts that form a premature termination codon and subsequently elicit the nonsense-mediated decay (NMD) pathway. It is estimated that 1/3 of genetic disorders and cancers could be the result of PTC containing transcripts. Accordingly, we have found 24 total and 21 unique mutations in patients with CPSID that result in stop codon formation, 19 of these unique mutations form PTCs, defined as such because they are not present in the last exon. This data was collected from a mutation screen that resulted in the detection of 153 total mutations, illustrating that PTC formation occurs in 15.7% of all mutations determined in our lab to be associated with CPSID. PTCs were generated from point mutations that created a stop codon directly as well as numerous single and multiple base pair insertions and deletions. Some PTC formation also occurred as the result of splicing defects. This data supports the evidence that many genetic mutations do not affect the protein product but rather the processing of the transcript, meaning that mature proteins are not always produced. Examining NMD in a growing number of diseases like CPSID could provide evidence that this is a prevalent mechanism leading to genetic deficiencies. This finding highlights the increasing importance of RNA processing defects in molecular pathology.

The Relationship between UDP-Galactose 4-Epimerase Activity and Galactose Sensitivity in Yeast. *J. Wasilenko, J. Fridovich-Keil.* Human Genetics, Emory University, Atlanta, GA.

Epimerase-deficiency galactosemia is an autosomal recessive disease that results from the impairment of human UDP-galactose 4-epimerase (hGALE), the third enzyme in the Leloir pathway of galactose metabolism. While severe or generalized GALE-deficiency has been reported in a small number of patients, the vast majority of patients have an apparently benign peripheral form of the disease in which impairment is restricted to circulating red and white blood cells. Patients with intermediate forms of hGALE-deficiency have also been reported. Like transferase-deficiency galactosemia, the underlying basis of pathophysiology in epimerase-deficiency galactosemia remains unknown, although a build-up of galactose metabolites has been implicated in both forms of the disease.

We are using a yeast model system to study the functional impact of GALE deficiency. Previously we have demonstrated that yeast that are GALE (*gal10*) deficient will not grow in the presence of galactose, despite the presence of another metabolizable carbon source. Here we have introduced a doxycycline-repressible promoter just upstream of the *GAL10* open reading frame in order to modulate its expression. Growth of these cells in medium containing different levels of doxycycline results in differential GALE expression, and differential galactose sensitivity. By characterizing these yeast, we are therefore able to explore the relationship between GALE impairment, accumulation of galactose metabolites, and growth in the presence of galactose.

The spectrum of mutations in *mut* methylmalonic aciduria and identification of a common Hispanic mutation and haplotype. *K.M. Niles*², *L. Worgan*¹, *J.C. Tirone*^{1,3}, *A. Hoffman*¹, *K. Morgan*^{1,3}, *A. Verner*⁴, *A. Sammak*⁴, *P. Lepage*⁴, *D. Rosenblatt*^{1,2,3}. 1) Department of Human Genetics, McGill University, Montreal, QC, Canada; 2) Department of Biology, McGill University, Montreal, QC, Canada; 3) Department of Medicine, McGill University, Montreal, QC, Canada; 4) McGill University and Genome Quebec Innovation Centre, Montreal, QC, Canada.

Cobalamin non-responsive methylmalonic aciduria (MMA, *mut* complementation class) is an autosomal recessive disorder resulting from mutations in the methylmalonyl CoA mutase (MCM) gene (*MUT*). In order to better understand the spectrum and ethnic origin of mutations causing MMA, the *MUT* gene was sequenced in 160 patients with MCM deficiency. Genomic DNA was extracted from patient fibroblasts and each of the 13 exons of the *MUT* gene was amplified by PCR and sequenced. Sequence analysis identified mutations in 96% (308/320) of alleles. Both mutations were identified in 152 patients, one mutation in four patients and no mutations in four further patients. 115 different mutations were identified. 82 of these mutations were novel, adding to the 54 *MUT* mutations that have been previously reported. Mutation types identified were missense (61/115, 53%), small deletions and insertions (25/115, 21%), nonsense mutations (19/115, 17%) and splice site mutations (10/115, 9%). Although many mutations were specific to an individual family, ethnic specific mutations were also identified. The previously reported G717V mutation was seen in 44% (12/27) of Black patients and several other mutations (G94V, G623R, T370P, F174S, F573S, G284X) were identified only in Black patients. There were two mutations seen only in Asian patients, G427D and G544X. A novel mutation in exon 2, R108C, was identified in 60% (16/27) of Hispanic patients. Sixty SNPs within a 134 kb region incorporating the *MUT* gene were examined by genotyping. This data demonstrated that the Hispanic patients with the R108C mutation share a common haplotype, distinct from other Hispanic patients and controls. Three other mutations (G94R, insertion A 1023 and A324T) were also seen exclusively in Hispanic patients.

Haplotypes of the Human Acid -Glucosidase Gene: In normal and individuals with Pompe Disease. *R. Pomponio, J. Marino, M. Phipps, R. Palmer.* Clinical Lab Science, Pharmacogenetics, Genzyme Corporation, Framingham, MA.

Mutations in the human Acid -Glucosidase (GAA) gene lead to absent or severely dysfunctional lysosomal Acid -Glucosidase. These genetic defects result in the progressive accumulation of glycogen in various tissues and organs. Attempts at establishing genotype-phenotype correlations through mutation analysis have been made in the study of the Pompe patient populations, but in many cases, individuals from the same family with the same mutations have been found to have discordant clinical progression. Allelic variation, synonymous and non-synonymous has been observed within and between racial and ethnic groups. However, it has always been unclear whether allelic variation and presence of particular mutations correlate to clinical phenotype. Molecular genetic analysis of the GAA gene was carried out for the identification of genetic changes in individuals with Pompe Disease (Glycogen Storage Disease type II). Sequence data was obtained from the study of 19 Pompe patients (15 infantile-onset, 3 late-onset), their parents, and a collection of normal individuals representative of different racial and ethnic groups and a haplotypes were determined based on a panel of 59 SNP markers identified in and between the GAA gene. We present our finding that there are at least 6 major haplotype groups in the normal population which are representative of the racial and ethnic backgrounds studied, as well as other distinct SNPs and haplotypes which serve as the genetic background markers specific for particular mutations.

Mutation of genes involved in CoQ10 biosynthesis pathway in patients with coenzyme Q10 biosynthesis defect and mitochondrial disorder. *J. MOLLET¹, D. SCHLEMMER¹, L. VAN MALDERGEM², A. MUNNICH¹, A. ROTIG¹.*
1) INSERM U393, Hopital Necker, Paris, France; 2) Centre de Genetique Humaine, Loverval, Belgium.

CoQ10 plays a pivotal role in the mitochondrial respiratory chain as it distributes electrons between the various dehydrogenases and the cytochrome segments of the respiratory chain. We have found CoQ10 deficiency in six patients from three unrelated families. In the first two families the non-consanguineous parents had two affected children while parents in the third family were consanguineous. Clinical presentations of the patients differed from one family to another. Patients from the first family presented retinitis pigmentosa, cataract, deafness, renal failure, cardiomyopathy and ataxia. Patients from family two had hypotonia, deafness and Leigh syndrome. Patients from the third family presented neonatal hyperlactatemia and died in the first days of life. Enzymological studies of the respiratory chain showed that quinone-dependent activities (complex I+III, complex II+III, glycerol-3-P cytochrome c reductase) were in the lowest control values whereas all respiratory chain complexes showed normal activities. LC/MS/MS quantification of quinones on cultured skin fibroblasts or muscle revealed a severe CoQ10 depletion in all patients (<15 nmol/g protein, control values 117+45). Ubiquinone is synthesized via a very complicated pathway, most genes of which have been identified in yeast. The human counterparts of these genes have been identified by sequence homology and mapped. We have tested 10 candidate genes encoding enzymes of CoQ10 biosynthesis pathway by studying the segregation of microsatellite flanking markers in the three families. Several genes have been excluded in all families (MVK, FPP, TPRT, COQ3, COQ4, COQ5, COQ8). We identified a homozygous mutation in one of the remaining genes in the patient born to consanguineous parents. This mutation is a one base pair deletion leading to a premature stop codon.

Exclusion of 17 COX subunits and assembly genes in cytochrome c oxidase deficiencies. *E. ZINOVIEVA*¹, *C. BOUCHET*², *S. LEBON*², *J.P. BONNEFONT*², *A. MUNNICH*¹, *A. ROTIG*¹. 1) INSERM U393, Hopital Necker, Paris, France; 2) Service de Genetique, Hopital Necker, Paris, France.

Cytochrome c oxidase (COX) deficiency is one of the most frequent causes of respiratory chain disorder in childhood and is a clinically heterogeneous condition. COX is the terminal electron acceptor of the respiratory chain and is composed of 13 different subunits. Three of them are encoded by mitochondrial genes and the other 10 by nuclear genes. Moreover, several proteins of nuclear origin are required for maturation and/or assembly of these subunits. Consanguinity of the parents in several families or recurrence of the disease is highly suggestive of an autosomal recessive mode of inheritance. We have identified several consanguineous and/or multiplex families with isolated COX deficiency. The clinical presentation was very different among families (encephalomyopathy, cardiomyopathy, Leigh syndrome, fatal and begin infantile myopathies, myoglobinuria) suggesting a genetic heterogeneity. We have tested 17 genes encoding various isoforms of COX subunits as well as 11 genes encoding COX assembly proteins by studying the segregation of microsatellite markers flanking the candidate loci. DNA from the patients only was studied. Non haploidentity and/or heterozygosity at the candidate loci allowed us to exclude 87% of COX subunits genes and 91% of COX assembly genes. Analysis of other candidate genes is currently under investigation. This work suggests that there is no major gene for COX deficiencies among candidate genes tested in our study.

Developing disease stage markers in late-onset metachromatic leukodystrophy. *K. Bjoraker, C. Boys, S. Pongonis, L. Charnas, D. Loes, C. Peters, W. Krivit, E. Shapiro.* Dept Pediatrics, Univ Minnesota, Minneapolis, MN.

Metachromatic leukodystrophy (MLD) is an autosomal recessive lysosomal neurodegenerative disease caused by the deficiency of the enzyme arylsulfatase-A (22q13.31). Sulfatide accumulation leads to progressive demyelination, dementia, and death. Our purpose was to identify disease stage markers (historical, neuropsychological, neuroradiological) in late onset MLD. With the advent of new treatments determination of stage of disease is increasingly important as success depends on early disease stage diagnosis. Methods: 31 subjects (18 females) over age 10 years were evaluated between 1989 and 2004 at the University of Minnesota for consideration of hematopoietic stem cell transplant. Measures: History- age of initial symptom onset, initial psychiatric diagnosis, most recent diagnosis prior to MLD diagnosis, years from symptom onset to psychiatric diagnosis, and years from symptom onset to MLD diagnosis. Neuropsychological: Cognition, attention, verbal memory, visual spatial, language, executive functions. MRI Baseline: Loes Score with a possible score of 0 to 26 (a demerit scale of number of abnormal brain regions). Results: A lengthy period to MLD diagnosis is almost universal and is preceded by ADHD and psychiatric misdiagnoses. Initial diagnosis: 61% had ADHD or mood disorders, 13% had neurological disorder, and none had psychosis or conduct symptoms. In contrast, immediately prior to MLD diagnosis, 75% were diagnosed with psychosis or severe conduct disorders. Symptoms progress from mild/moderate attentional symptoms to severe frontal presentation that includes schizophrenia and severe conduct disorder. Deficits were found in adaptive behavior, verbal memory, and verbal fluency and relative strengths in reading and receptive vocabulary. A significant relationship was found between neurological disease/MRI and specific neuropsychological correlates (attention, verbal memory, visual spatial, perception, math achievement). Conclusion: A pattern of onset of attention and psychiatric symptoms in a previously healthy individual with deficits in memory, attention, and spatial perception signals the need for a thorough medical investigation.

Program Nr: 1766 from the 2004 ASHG Annual Meeting

The Pompe Registry. *B. Byrne*¹, *P. Kishmani*². 1) University of Florida, Gainesville, FL; 2) Duke University Medical Center, Durham, NC.

Pompe disease (glycogen storage disease type II) is a progressive and often fatal muscle disease with a spectrum of severity from early presentation in infants to adulthood. The underlying pathology is a deficiency or dysfunction of acid alpha-glucosidase, a lysosomal enzyme that lysosomal glycogen to glucose within lysosomes. Due to the rarity of the disease, heterogeneity of symptoms, and multiple organ systems affected the collection of patient-level longitudinal natural history data is only possible through a broad effort across many physicians treating the disease. To that end, the Pompe Registry was created to collect patient baseline demographics as well as musculoskeletal, respiratory, cardiac, and GI manifestations over time. Patient data is confidential and de-identified and is available to the treatment center for real time analyses of disease progression. In addition, aggregate data of all patients in the Registry will be available to assess trends in disease states and establish management recommendations. To date, over 25 individual physicians have agreed to participate in the Registry. 5 patients are enrolled. These first patients are highly heterogeneous in their presentation and may be indicative of the continuous spectrum of phenotypes observed in Pompe, which differ in terms of age of onset, extent of organ involvement, and rate of symptomatic progression. The Pompe Registry is an international collaboration that attempts to increase the understanding of this rare disease and, therefore, to potentially improve management.

Glucosamine for the management of behavior in mucopolysaccharidosis type III (MPS III). *J. Clarke, E. Kerr, N. Poplawski.* The Hospital for Sick Children, Toronto, Ontario, Canada.

BACKGROUND: An open-label pilot study in 10 children with MPS III showed treatment with oral glucosamine hydrochloride lead to statistically significant improvements in parental ratings of behaviour. **AIM:** To determine if treatment with glucosamine has a beneficial effect on the behaviour of children with MPS III. **METHOD:** A randomised, double-blind, placebo-controlled study consisting of baseline estimate of mental age, functional independence and behaviour, with repeat assessment of behaviour after 6 weeks therapy with glucosamine or placebo (lactose). Mental age and functional independence were estimated using the Scales of Independent Behaviour-Revised (SIB-R). Behaviour was assessed using the Aberrant Behaviour Checklist-Community (ABC) and the long forms of the Conners Parent Rating Scale (CPRS) and the Conners Teacher Rating Scale (CTRS). **RESULTS:** 32 children have been recruited. Completed data are available for 24 (11F, 13M, mean age 8y 3m, range 4y to 14y8m), 14 with MPS IIIA, 6 IIIB, 1 IIIC and 3 IIID. Prior to unblinding parents were asked what they thought their child received. 10 (42%) thought glucosamine (all reported improved behaviour and 5 requested therapy continue) and 14 placebo (none reported improved behaviour). Analysis of the CPRS divides the children into 2 groups; improved behaviour (N=14) and no improvement (N=10). Improvement in the CPRS shows correlation with the ABC irritability score (11/14, 79% improved) and parental opinion (8/14, 57% improved) but not the CTRS (2/14, 14% improved). **CONCLUSION:** Interim analysis, prior to unblinding, suggests glucosamine may have a beneficial effect on the behavioural disturbance of MPS III. Analysis of the data following unblinding will be revealing. If it confirms glucosamine is beneficial it will pave the way for a new, safe treatment for children with MPS III and behavioural disturbance.

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An automated HPIEC system for the diagnosis of the mucopolysaccharidoses. *P.R. Clements, V.J. Muller, M. Fietz, J.J. Hopwood.* National Referral Laboratory, Genetic Medicine, Women's & Children's Hospital, North Adelaide, SA, Australia.

The diagnosis of the mucopolysaccharidoses by enzyme assay is crucial for the correct identification of the MPS subtype as there can be considerable clinical overlap. Measurement of enzyme activity is performed in our laboratory after a urine screen has been used to determine the identity of the stored substrate [1]. Enzyme assays are performed using radiolabelled substrates which are chemically derived from glycosaminoglycans [2-5]. After incubation of the substrate with extracts from patient cells or tissues, substrate and product are separated by an HPLC system with autoinjector. Separation is by fast flow (4mL/min) ion exchange chromatography (HPIEC) using an ammonium sulfate gradient coupled with on-line scintillation detection. The system has a turnaround time of eight minutes per sample. Assays performed routinely in our laboratory using this system include those for MPS types I, II, IIIA, IIIC, IIID, IVA, VI. (Type VII is measured using a fluorogenic substrate while IIIB assay was by fluorogenic substrate or by separation with paper electrophoresis). The system is reliable in sample recoveries, is not labour intensive and provides a rapid and definitive result. Our experience is that the natural substrates provide accurate discrimination between the MPS types. Provision of these tests enables us to act as a single centre for diagnosis of the full range of MPS disorders. A summary of the NRL experience using this system since 1995 includes the diagnosis of 370 cases of MPS and the performance of 91 prenatal tests. 1. Hopwood JJ & Harrison JR (1982) *Anal. Biochem* 119:120-127. 2. Hopwood JJ and Elliot H. (1981) *Clinica Chimica Acta* 112: 55-66. 3. Hopwood JJ (1979) *Carbo. Res.* 69:203-216. 4. Hopwood JJ & Elliott H *clin.Chim.acta* (1982) 123: 241-250. 5. hopwood JJ, Elliott H, Muller VJ & Saccone GTP *Biochem. J.* (186) 234: 507-514.

The Arg482His mutation in the -galactosidase gene is responsible for a high frequency of GM1-gangliosidosis carriers in a Cypriot village. *T.K. Georgiou¹, G. Stylianidou², V. Anastasiadou², A. Caciotti³, Y. Campos⁴, E.*

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GM1 gangliosidosis is a lysosomal storage disorder caused by deficiency of -galactosidase. It is mainly characterized by progressive neurodegeneration and in its most severe infantile form it leads to death before the age of four. The GLB1 gene gives rise to two alternatively spliced mRNAs which encode the -galactosidase and the elastin binding protein (EBP). The diagnosis of two patients with the infantile form of GM1 gangliosidosis and eleven carriers in a small mountainous village in Cyprus prompted us to carry out a study in order to establish the frequency of carriers in the village and identify the mutation(s) involved. Carrier detection was initially based on the measurement of -galactosidase activity in leucocytes. Among 85 random samples from the village, 10 were classified as carriers. Sequencing of the GLB1 gene in a Cypriot patient identified the missense mutation c.1445G>A (p.Arg482His) in the homozygous state. Seven of the ten carriers identified using the enzyme assay were found to carry the same mutation by NspI restriction enzyme analysis. The three individuals who were negative for the c.1445G>A had borderline enzyme results and were probably wrongly classified as carriers. The frequency of GM1 gangliosidosis carriers in this village is approximately 8% (1:12). Northern blot analysis in skin fibroblasts from one patient showed mRNA of normal size and quantity, and expression studies in COS-1 cells showed no enzyme activity. Western blot analysis showed a marked decrease of the 64kDa mature form of the enzyme protein and reduced amount of the 67kDa EBP. Our results indicate that the c.1445G>A mutation, which is responsible for all GM1 gangliosidosis alleles in this Cypriot village, affects the stability of the mature -galactosidase protein.

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The Lysosomal Disease Research Network. *J. Hegland¹, C. Whitley¹, J. Barranger², C. Eng³, G. Grabowski⁴, J. Muenzer⁵, J. OBrien⁶, G. Pastores⁷, E. Shapiro¹, R. Steiner⁸, W. Wilcox⁹, and the Lysosomal Disease Organizing Committee.* 1) University of Minnesota, Minneapolis,MN; 2) University of Pittsburgh, Pittsburgh,PA; 3) Baylor College of Medicine, Houston,TX; 4) Children's Hospital Research Foundation, Cincinnati,OH; 5) University of North Carolina, Chapel Hill,NC; 6) Mayo Clinic, Rochester,MN; 7) New York University, New York,NY; 8) Oregon Health and Science University, Portland,OR; 9) Cedars-Sinai Medical Center, Los Angeles,CA.

A network devoted to promote and facilitate basic, translational and clinical research in lysosomal diseases (LDs) has recently been established. The network is a scalable multi-center consortium of geographically distributed expert medical centers, patient support and corporate partners. The infrastructure, mission and charter were developed through organizational committees; the collaborating medical centers, pharmaceutical and patient support organizations will direct the activities of the network through participation in the Steering Committee and Advisory Board. The network is advised through expertise-oriented councils (ie, Council of: Clinical Investigators; Scientists; Patient Advocates; Industry Representatives and Laboratory Diagnosticians). The first annual scientific meeting was held May 13-15,2004 and provided a multidisciplinary forum to present and discuss basic and clinical research discoveries in LDs, and related treatment, ethical, and quality of life issues. Plans for the next annual meeting (May 19-20,2005) are underway. The network is currently developing longitudinal studies to understand the natural history of LDs and treatment outcomes. Long term network goals include 1)development of an information management infrastructure to encourage integration of shared clinical experience and relevant longitudinal studies in LDs, 2)promotion of programs to train LD clinical investigators, and 3)development of a web site to facilitate professional and public education, timely network communication, and a data entry mechanism. Interested parties are encouraged to become involved (hegla005@umn.edu). Organizational support has been provided by NIH, NINDS and ORD, NS49950.

Decreased Quality of Life in Women With Fabry Disease. *R. Hopkin¹, N. Jansen¹, L. Bailey¹, M. Yi².* 1) Cincinnati Children's Hosp; 2) Univ Cincinnati Med School.

Purpose: Fabry disease is an X-linked lysosomal storage disorder that is known to have a major impact on the lives of affected men. It is not known how significantly Fabry disease impacts the lives of heterozygous women. The aim of this study is to determine if there are significant differences in quality of life between women heterozygous for Fabry disease and women in the general population. **Methods:** The SF-36 was given to obligate heterozygous women identified at lysosomal disease centers in the US and to all members of the FSIG. Participants were recruited based upon family history regardless of symptoms. Over 150 questionnaires were returned. Preliminary analysis of 88 questionnaires has been performed using the RAND scoring system with the WHI as a control population (n>8300). An unpaired t-test evaluated the statistical differences between the two groups. CID value was used to evaluate the clinical importance of differences between the groups where >0.80 represents high impact and >0.50 a moderate effect. **Results:** The difference between each of the mean scores was statistically and clinically significant in all 8 HR-QOL categories with p<.0001. and CID>0.50.

Hlth Meas	WHI	Fabry	CID value
Gen Health	76.7+16.2	42.8+24.2	2.09
Body Pain	77.3+21.8	60.8+27.4	0.76

Conclusion: Women with Fabry disease reported significantly lower quality of life in all 8 areas of functioning (e.g. general health and bodily pain - see table). The quality of life profile more closely resembled that of women with Multiple Sclerosis than the general population. These results suggest that Fabry disease impacts the health of heterozygous women more than previously appreciated, and highlights the need for additional studies to develop treatment protocols for women with Fabry disease.

***C.elegans* has only one alpha-galactosidase A and alpha-N-acetylgalactosaminidase ortholog.** M. Hrebicek, J. Hujova, R. Dobrovolny, D. Holanova, B. Asfaw, H. Poupetova, J. Ledvinova, M. Kostrouchova, J. Sikora. Institute of Inherited Metabolic Disorders, Prague, Czech Republic.

Human alpha-galactosidase A (GAL) and alpha-N-acetylgalactosaminidase (NAGA) are true paralogs sharing a common ancestral gene. Deficiencies of these enzymes cause human lysosomal storage disorders - Fabry (GAL deficiency) and Schindler (NAGA deficiency) diseases. BlastP searches for orthologs of human GAL and NAGA revealed a single *C.elegans* gene (CeGAL/NAGA) with significant homology to both human genes. We elucidated gene organization of CeGAL/NAGA by cDNA sequencing including 3' and 5' UTR. We performed phylogenetic analyses and homology modeling of CeGAL/NAGA based on 3D structure of chicken NAGA. The GAL and NAGA activity measurements in *C.elegans* mixed culture homogenate revealed presence of both enzyme activities. In contrast to other organisms, the GAL activity in the worm was completely inhibited by N-acetylgalactosamine - a NAGA inhibitor. To study the expression of CeGAL/NAGA in *C.elegans*, we created C-terminal GFP fusion of the whole CeGAL/NAGA ORF under the control of its natural promoter. Presence of CeGAL/NAGA-GFP transgene was confirmed at the level of gDNA, cDNA and protein. pH-dependent GFP fluorescence was observed in membrane-bound compartment of coelomocytes only after treatment with concanamycin A and NH₄Cl, a finding supporting lysosomal localisation of the expressed protein. Immunofluorescence detection of GFP fusion protein showed transgene expression in muscle, intestinal cells and vesicular compartments of coelomocytes. RNA interference assays directed against the CeGAL/NAGA have not revealed any abnormal morphological phenotype, however a biochemical phenotype was observed. The activities of GAL and NAGA decreased in a similar manner in interfered worms, usually by tens of percent of activity of controls. The hypothesis of a single ortholog in *C.elegans* was further supported by phylogenetic, homology modeling and biochemical analyses. RNA interference does not lead to a successful model of Schindler and Fabry diseases in *C.elegans*. However, a deletion mutant of CeGAL/NAGA may provide an interesting model relevant for both disorders.

Home enzyme replacement therapy (ERT) with Replagal for Fabry disease (FD) in Nova Scotia. *K. LeMoine, M. West, C. Simms, F. MacLennan.* Medicine, QE II Health Sciences Centre, Halifax, NS, Canada.

FD is a rare X-linked lysosomal storage disease due to deficiency of α -galactosidase A activity (α -gal). Nova Scotia has one of the world's largest FD kindreds with over 66 adults. However as the province is sparsely populated, FD patients may have to travel up to 4 hours by car to receive enzyme replacement therapy every 2 weeks in Halifax, the regional tertiary care centre. Home ERT can be safely delivered either by a visiting nurse or by the patient. We report our experience with both in Nova Scotia over the past for years.

Eight adult male patients (age 27-52, mean 38.6 years) with FD had received ERT (Replagal 0.2 mg/kg iv every two weeks) in a clinic trial (TKT015) for at least 6 months; none had ever had an infusion reaction. Home infusion was carried out on all patients for 18 months by a visiting nurse for 312 treatments. There were no infusion reactions or other problems.

Home self infusion of ERT has been successfully carried out in 2 patients with FD in Nova Scotia. Patients and their support person were trained for 3 consecutive sessions in the hospital by a nurse educator. Skills taught included hand washing, sterile technique, iv tubing and medication preparation, drug administration, detection of adverse effects and safe disposal of medical equipment. Once patients were able to show these skills in hospital, they were sent home to begin home self infusion therapy. A follow up telephone call is made after each infusion of Replagal to identify any issues. To date there have been no adverse events in the two patients trained. Home ERT is well tolerated and less expensive than that given in larger regional hospitals. It is a safe, well-tolerated and convenient way of providing ERT.

Study of Natural History of Juvenile GM2 Gangliosidosis - Analysis of 20 patients. *G. Maegawa¹, M. Tropak¹, D. Mahuran¹, T. Stockley¹, J. Callahan¹, F. Kok², C. Vasques², R. Rosemberg², M. Vainzof², R. Giuglianti³, C. Barros³, M. Burin³, J. Clarke¹.* 1) Div Clin. & Metab. Genetics, Metab. Program, Research Institute, Molecular Genetics Lab., Hospital for Sick Children, Toronto, ON, Canada; 2) Div of Neuroped., Centro do Genoma Humano, USP, SP, Brazil; 3) Medical Genetics Service, HCPA, Porto Alegre, RS, Brazil.

Juvenile GM2 gangliosidosis (jGM2) is a rare neurodegenerative disorder characterized by progressive ataxia, dystonia, pyramidal signs, dysarthria and psychiatric symptoms, caused by lysosomal beta-hexosaminidase A deficiency. We report a study of 20 patients from 14 unrelated families, 13 diagnosed with Tay-Sachs variant (TSV) and 7 with Sandhoff variant (SV). The mean age of onset was 5.5 years (1.5 to 17 years), 11 males and 9 females. The most common symptoms at onset were ataxia and speech problems which were found in all jGM2 patients. Interestingly, the mean of onset of gait ataxia and dysarthria were similar, 7.1 years and 6.9 years, respectively. However, the progression of the dysarthria was faster than the ataxic gait problems (1.8 years from mild to moderate dysarthria against 3.4 years from mild ataxic gait to a moderate ataxic gait needing a device). Psychiatric symptoms, such as psychotic episodes, aggressive or compulsive behaviour and also schizophrenia developed in 11 patients and were more common among SV patients (5/7). Eight patients developed seizures at a mean age of 5 years (2 to 7.5 years). Gastro-intestinal symptoms such as dysphagia, constipation and diarrhea were present in 15 patients (8/13 in TSV and 7/7 in SV). Acroparesthesia (3/7) and hearing problems (3/7) were also more common in patients with SV. Among these 20 patients, five were considered as having an adult-onset of GM2. However a detail inquiry about the childhood of these patients revealed that they had motor signs and even abnormal behaviour during childhood and early adolescence. Therefore they are included at the study. The juvenile form of GM2 is described as homogeneous. However among these patients, we observed considerable clinical heterogeneity in the clinical presentation as well as in the progression of this condition.

A Cross-Sectional Survey Study to Collect Normative Data in Patients with Niemann-Pick B Disease. M.M.

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Niemann-Pick disease Type B (NPD-B) is a lysosomal storage disorder resulting from deficient acid sphingomyelinase activity and the accumulation of sphingomyelin. The clinical phenotype and spectrum of disease manifestations have been described only in a small number of patients. Fifty-nine patients (31 M, 28 F; mean age 22.6y, age range 7 to 65y) with NPD-B underwent comprehensive clinical evaluations. The most common symptoms at diagnosis were splenomegaly (78%), hepatomegaly (73%), and respiratory (20%). Mean liver and spleen volumes were 1.87 and 11.14 multiples of normal. Mean weight and height Z scores were -0.77 and -1.29. Growth retardation was common in children and adolescents. The most common laboratory findings were elevated chitotriosidase (95%), liver function tests [SGOT (51%), SGPT (50%), alkaline phosphatase (43%), and bilirubin (33%)], and IGF-1 binding protein (35%); abnormal fasting lipid profile [elevated triglycerides, (62%), LDL (46%), cholesterol (41%), and low HDL (74%)]; and low platelets (53%) and IGF-1 (35%). Macular haloes or cherry red spot were present in 25% of patients. Most patients underwent both radiographic and pulmonary function evaluations. CXR and HRCT revealed interstitial lung disease (ILD) in 90% and 96% of patients, respectively. The mean DLCO was 60% of predicted (n=43) with a trend for increasing values as the HRCT ILD severity score decreased ($R^2 = 0.1460$, $p=0.0114$). Of the 48 different mutations identified, R608del was the most common (25% of all alleles). In summary, hepatosplenomegaly, liver dysfunction, thrombocytopenia, lipid abnormalities, short stature, and pulmonary involvement are common features of NPD-B, although there is considerable clinical and molecular genetic heterogeneity among affected patients.

The MPS I Registry: Centralised data collection to delineate the natural history and treatment of MPS I. G. Pastores, on behalf of the MPS I Registry Boards of Advisors. NYU School of Medicine, New York, NY.

Mucopolysaccharidosis I (MPS I) is a chronic disorder caused by the deficiency of the lysosomal enzyme -L-iduronidase. The accumulation of glycosaminoglycans (GAG) in many tissues leads to a heterogeneous disease with a clinical spectrum ranging from severe to attenuated. The primary objectives of the MPS I Registry are to characterize and describe the MPS I patient population and evaluate the long-term effectiveness and safety of specific therapies. The MPS I Registry is a voluntary, multi-center observational program established to monitor the outcomes of all patients with a confirmed diagnosis of MPS I. Data will be analyzed to address questions related to diagnosis, disease course, and management. As of April 2004, 53 patients, from Europe, U.S. and Brazil, have been enrolled. The current age of these patients ranges from 5 months to 46.3 years (median age of 6.1 years). The age at diagnosis ranges from prenatal to 26 years (median age of 1.5 years). Sixty-six percent (66%) of the patients were characterized as having the severe form of the disorder. The majority (70%) of the patients are currently on enzyme therapy (Aldurazyme[aronidase]), and the remainder have undergone hematopoietic stem cell transplantation (HSCT) (2%) or not been treated with either approaches (19%). In addition, 9% of the patients have been treated with a combination of Aldurazyme and HSCT. Data are captured on many of the systems impacted by the disease, including CNS, cardiac, respiratory, gastrointestinal, musculoskeletal, ocular, and auditory. Height, weight, head circumference and urinary GAG are part of the recommended assessments. As of June 2004, 96 patients have been enrolled and further analyses are planned. Initially, the MPS I Registry will provide descriptive data on the baseline clinical status of patients, delineating the pattern and severity of disease at enrolment. Over time, the MPS I Registry will utilize longitudinal data to focus on specific aspects relevant to the diagnosis and management of MPS I, with the anticipated overall outcome of improving the management of patients.

The effects of anti-psychotic drugs on adult Tay-Sachs disease and implications for treating associated psychoses.

B.A.J. RIGAT, A. Mocon, R. Geist, D. Mahuran. The Hospital for Sick Children, Toronto, Ontario, Canada.

Adult Tay-Sachs disease (ATSD) is characterized as a deficiency in activity of hexosaminidase A (Hex A) resulting in intralysosomal accumulation of GM2 ganglioside. ATSD and other variants of Tay-Sachs disease exhibit an autosomal recessive pattern of inheritance for mutations of the HEXA gene encoding the α -subunit of Hex A. Psychiatric abnormalities affect approximately 40 percent of ATSD patients and present as the most prominent and impairing symptoms. However, clinical treatment of ATSD with anti-psychotic drugs is controversial. It has been shown that such drugs are lysosomotropic, inducing lysosome lipid storage and enzyme secretion, thereby potentially exacerbating the disease phenotype. We have examined the effects of the weakly basic antipsychotic drugs imipramine, risperidone, olanzapine and quetiapine on various lysosomal enzymes in wild type and ATSD human fibroblasts. Western blots and enzymatic assays of dose-response studies indicate that, relative to a treated patients plasmatic drug concentration, only very high levels of imipramine decrease protein levels and enzyme activity in cells while correspondingly increasing the levels and activity of each in culture media. For all drugs examined, negligible effects were seen with drug concentrations of up to 10 fold times their respective therapeutic levels. For imipramine, we observe the presence of a concentration threshold at approximately 10 mM after which cell death occurs. This suggests the involvement of the lysosomal V-ATPase proton pump in the lysosomotropic effect. We hypothesize that the threshold level corresponds to the concentration of imipramine for which the proton pump can no longer compensate to maintain the functionally necessary low intra-lysosomal pH. We are investigating this hypothesis using concanamycin A, an inhibitor of the V-ATPase pump. Our data suggests that lysosomotropic effects of antipsychotic drugs are only apparent at hyperphysiological concentrations, not at the levels expected in patients' plasma. Therefore, the use of antipsychotic drugs in ATSD patients is unlikely to promote additional lysosomal enzyme secretion and lipid accumulation.

Novel reaction to Fabrazyme in an 8 year old male with Fabry disease: adverse event or natural history of pediatric Fabry? *S. Sastry, A. Ahmad.* Genetics and Metabolism, Children's Hosp of Michigan, Detroit, MI.

Fabry disease (FD) is a lysosomal storage disease caused by a deficiency in α -galactosidase A, which results in accumulation of globotriaosylceramide in vascular endothelial cells. Manifestations of FD include angiokeratomas, acroparesthesias, hypohydrosis, abdominal pain, renal failure, cardiomyopathy and stroke. In 2003, the FDA approved Fabrazyme for the use of enzyme replacement therapy (ERT) in FD. Mild to moderate adverse infusion associated reactions (fevers and rigor) have been reported during or shortly after infusion in adults. To our knowledge, delayed adverse reactions (>24hrs post infusion) have not been reported.

Symptoms of FD are progressive with onset as early as 3 yrs of age in boys. ERT is advocated in symptomatic children to reverse and/or prevent the sequelae of FD. Little information is available on tolerance of infusion in children. We present an 8 yr old male diagnosed at 18 months of age due to a positive family history. Symptoms of headache and overheating following physical exertion; associated with episodes of acroparathesia and hypohydrosis, started at 5 yrs of age. These were mild episodes, well controlled with Acetaminophen or Ibuprofen. ERT with Fabrazyme was initiated at 8 yrs of age. To date, he has had nine biweekly infusions with no adverse reactions during infusion. However, between 24 and 72 hours after his 2nd, 3rd and 4th infusions, he experienced episodes of fever up to 104F with lethargy, severe acroparathesias, headaches, abdominal pain and back pain. Each episode lasted about 48 hours and was significantly more severe than any episode experienced prior to initiation of ERT. There was no associated intercurrent illness or other inciting trigger. No further adverse events were noted during or following the remaining infusions. He now shows improvement in his clinical status with decreased severity and frequency of acroparathesia, increased ability to sweat and improved tolerance to exercise.

We hypothesize that adverse infusion related reactions can occur during and as far as 72 hours post ERT, but may be self limiting.

Gaucher Disease Therapy: Conditional liver expression of acid -glucosidase (GCase) improves visceral, but not CNS disease in mouse models. *Y. Sun, B. Quinn, G.A. Grabowski.* Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Gaucher disease is an autosomal recessive trait and the most common lysosomal storage disorder. A variant Gaucher mouse model (Enron) was created by generating mice with a GCase V394L point mutation (V394L) and a partial deficiency of prosaposin. Enron mice displayed large numbers of storage cells in the liver, lung, spleen and thymus. The major lipid stored was glucosylceramide (GC). To test the organoid therapeutic approach to the glycosphingolipid storage diseases, a tTA-responding human acid -glucosidase (hGCase) transgenic system was introduced into Enron mice. The expression of tTA-hGCase is under the control of liver-enriched activator protein promoter (LAP). RT-PCR analyses showed that hGCase mRNA was present in liver (high level) and lung (low level), and hGCase protein and activity were detectable in liver, lung and spleen. hGCase was at higher level in serum indicating the enzyme was secreted. In the absence of DOX, expression of hGCase reduced the number and size of storage cells and GC accumulation in the visceral tissues. hGCase expression in these mice can be turned off completely with DOX. The pathology became manifest in liver, lung and spleen within two months on DOX. These results demonstrate that high level expression of hGCase and reversed visceral pathologic lesions. The tTA-response hGCase system represents a powerful tool to facilitate evaluation of therapeutic approaches to the Gaucher disease and other lysosomal storage diseases.

Common Hexosaminidase B (HEXB) polymorphisms effect results of enzymatic carrier testing for Tay-Sachs disease. *HD. Vallance, T. Morris, B. Toh, M. Coulter-Mackie.* Pathology, Children's Hospital of B.C., Vancouver, BC, Canada.

Two brothers known to carry the 1278insTATC mutation in the Tay-Sachs (HEXA) gene were found to have normal serum %Hexosaminidase A (%HexA) enzyme activity, above the screening cut off for carrier detection yet in white blood cells (WBC) %Hex A was well within the carrier range. Total Hex and HexB enzyme activities were well below reference range. The biochemical profile was suggestive of combined inheritance of mutations in HEXA and B genes. Sequencing of the HEXB exons revealed that both brothers had 3 HEXB polymorphisms: I207V and 3' UTR delTG (cis) and S62L(trans). The delTG polymorphism has previously been reported to reduce HEXB mRNA. The allele frequencies in the Ashkenazi Jewish (n=72) vs. non-Jewish populations (n=104) were determined: I207V (11%;17%), delTG (13%;11%) and S62L (2.8%;3%) respectively. Given the high allele frequencies of these HEXB polymorphisms we undertook a retrospective analysis of serum (n=12) and WBC (n=11) enzyme results from non HEXB carrier controls and compared them with data from HEXB polymorphism carriers. In serum (n=2) and WBC's (n=1), delTG carriers had HexB levels 40% below the normal mean (P.001). In serum and WBC's, an I207V carrier had normal HexB but a HexA level 20% below the normal mean (P.001) suggesting that this polymorphism impairs formation of functional HexA (alpha / beta heterodimer). Interestingly, a I207V/delTG carrier had normal serum HexA but a 25% reduction of HexA in WBC's (P.001); a similar trend to the brothers described above. In summary, this is the first report of reduced HexA and HexB activities associated with common HEXB polymorphisms. The inheritance of the delTG mutation reduces HexB and has the potential to elevate %HexA. In addition, the combination and interaction of polymorphisms can further affect HexA as seen with I207V / delTG carriers and the reduction in HexA may only be apparent in WBC's and not serum. We hypothesize that this effect could potentially mask carrier status in serum for a HEXA mutation and may explain the normal %HexA in serum but low level in WBC's in the index family. This hypothesis is being tested with a larger sample size. Funded by Garrod Assoc.

A screening study for Fabry disease (FD) using whole blood filter paper technique in renal transplant (Tx) and dialysis patients in Nova Scotia (NS). *M. West¹, J. Medin², K. LeMoine¹, A. Poepl², T. Hewlett³, J. Dhar⁴.* 1) Dept Medicine, Dalhousie Univ, Halifax, NS; 2) University Health Network, University of Toronto, Toronto, Ont; 3) Dept Medicine, Cape Breton Regional Health Centre, Sydney, NS; 4) Dept Medicine, Yarmouth Regional Health Centre, Yarmouth, NS, Canada.

FD is a rare X-linked lysosomal storage disease due to deficiency of α -galactosidase A activity (α -gal). The prevalence of FD is reported as 1:50,000. NS has a large well-defined FD kindred with a prevalence estimated at 1:14,000. All 66 adult FD patients in NS have classic phenotype and uniform genotype A143P; they are descendants of a founder who emigrated from Montbéliard France to NS in 1750. A prospective screening study in NS of all adult patients (18 years old) with a renal Tx or who are on dialysis is being carried out to determine the true prevalence of FD as a cause of end stage renal disease and to look for previously unidentified cases of FD.

All NS dialysis or renal Tx clinic patients were approached for this study. Known FD patients were excluded: 3 renal transplant (2 cadaver and 1 living related donor), 0 dialysis patients. Consent was refused in 12.8%. 706 patients have been screened to date. Filter paper technique with dried whole blood spots was compared with plasma to measure α -gal. A subset of 10 hemodialysis patients had samples drawn pre and post dialysis to test for an acute influence of hemodialysis. Of 549 (77.8%) samples analyzed to date, 4 male hemodialysis patients have low α -gal (0.7%). None of these is known to have FD. No Tx or peritoneal dialysis patients had low α -gal. 2 of 2 tested have normal plasma α -gal; DNA mutational analysis is pending to confirm FD. There was no observed influence of hemodialysis on whole blood or plasma α -gal.

In conclusion, several NS hemodialysis patients with low α -gal were identified consistent with FD in a province-wide screening study; uremic inhibition of α -gal cannot be excluded. Whole blood filter paper technique was comparable with plasma α -gal. Whether the patients with low α -gal have FD requires further study.

-Mannosidase Deficient Mice: A Model of Lysosomal Storage Disease. *M. Zhu*¹, *K.L. Lovell*², *T.L. Saunders*⁴, *J.S. Patterson*³, *K.A. Friderici*¹. 1) Microbiol/Molec Genetics; 2) Neurology/Ophthalmology; 3) Pathobiol/Diag Invest, Michigan State University, East Lansing, MI; 4) Transgenic Animal Model Core, University of Michigan, Ann Arbor, MI.

-Mannosidase is a lysosomal enzyme that is involved in the degradation of Asn-linked glycoproteins in lysosomes. The deficiency of this enzyme causes an autosomal recessive inherited disorder, -mannosidosis. This lysosomal storage disease is characterized by the intracellular accumulation of small oligosaccharides in many cell types. In human cases of -mannosidosis the clinical symptoms, which include mental retardation, hearing loss, frequent infections and behavior disturbance, can be relatively mild and little is known about the pathology. However, in the two ruminant animal models (cows and goats) the clinical presentation is severe at birth with skeletal deformation, neurological deficits and neonatal demise. Pathology includes cytoplasmic vacuoles in many organs and in CNS, and severe myelin deficits in the cerebral hemispheres. To explore and clarify the pathogenesis of this disease and to establish potential therapeutic strategies, we have generated the first mouse model with targeted disruption of the -mannosidase gene by homologous recombination in a mixed C57B6/129J mouse background. Homozygous mutant animals exhibit -mannosidase enzyme deficiency. General appearance and growth of the homozygous mutant mice is similar to heterozygous and wild-type littermates. Morphological characterization of the homozygous mutant animals reveals cytoplasmic vacuolation in neurons as well as visceral organs including thyroid gland, kidney, epididymis, and liver. The morphological lesions resemble those found in the two ruminant models, but with less severe expression. The neuronal vacuolation is selective, with pyramidal cells in the cerebral cortex especially involved. Severe hypomyelination is not apparent. This mouse model might be closer to the human -mannosidosis case; it will be a very useful tool for studying the phenotypic variations in different species and may help to facilitate a potential treatment for lysosomal storage diseases.

Novel *SLC39A4* Mutation in Kindred with Acrodermatitis Enteropathica. *Y. Anikster*¹, *N. Gal*¹, *J. Abu-Moch*³, *G. Paret*², *M. Rubinshtein*², *Y. Bujanover*⁴, *A. Vardi*². 1) Metabolic Disease Unit, Safra Childre's Hospital, Tel Hashomer, Ramat Gan, Israel; 2) Pediatric Intensive care, Safra Childre's Hospital, Tel Hashomer; 3) Kupat-Holim Clinic; 4) Pediatric, Safra Childre's Hospital, Tel Hashomer.

Acrodermatitis Enteropathica (AE) is a rare autosomal recessive disorder affecting early infancy. The triad of dermatitis, alopecia and diarrhea is its diagnostic hallmark. Other common manifestations are mental change, failure to thrive, nail dystrophy and immune system dysfunction. Similar clinical manifestations are observed with zinc deficiency from any cause. Recently, the human gene encoding an intestinal zinc transporter of the ZIP family, *SLC39A4*, was identified as the mutated gene in AE.

We studied a 4-year-old girl who is the first child of healthy first-cousin parents. She was hospitalized for an investigation of congestive heart failure and lymph node enlargement. Low alkaline phosphatase was found in the routine blood test. After a more thorough study of her medical history, we noticed that she was treated with zinc supplements for dermatitis at the age of nine months. Moreover, she has a 13-year-old boy cousin who was diagnosed with zinc deficiency and still has unhealed scars on his face.

DNA was extracted from peripheral blood samples from both children and their parents. Genomic DNA of the patients was then PCR amplified for each exon of the *SLC39A4* gene and analyzed by direct nucleotide sequencing. We identified a novel 1223 C deletion, this frame shift at amino acid A408 created after 76 amino acid, a stop-codon (A483X). This deletion in exon 7 destroyed a BsuR1 restriction site in the mutant amplicon and created a new Aci I restriction site. Using these restriction enzymes, we checked 30 members of this Arab-Muslim genetics isolate and found 17 carriers for this novel mutation.

Our results allow prenatal diagnosis and carrier detection in these large kindred and widen the clinical spectrum of AE.

Creatine Transporter Deficiency. *C. Bellini, M.C. Schiaffino, L. Costabello, U. Caruso, E. Bonioli.* Dipartimento Pediatria, Università Genova, Istituto G., Genova, Genova, Italy.

The patient was born at 40-week (uneventful pregnancy; weight 3370 g). His non consanguineous parents, brother, and sister are healthy. Increasing psychomotor retardation, mild failure to thrive, and recurrent vomiting was observed as of the third month of life. He came to our observation (age 21 months): there was generalized hypotonia and alternate exotropia with right predominance. EEG showed slow diffuse hypersynchronisms with abnormal multifocal spikes. Polygraphic recordings showed diffuse generalized parosistic "bouffes" with simultaneous eye and upper limb myoclonic movements. Cranial CT showed hypodensity of caudal nucleus and cerebral MRI showed a hyperintense signal in the posterior periventricular white matter and in the posterior part of the basal ganglia (T2-weighted images). Hematological, hormonal, and aminoacids in plasma and urine examinations were normal, karyotype (750 band level) and molecular evaluation for FraX and Angelman syndromes were negative. Plasma creatinine levels were persistently low (0.26-0.32 mg/dl). Urinary guanidinoacetate was within the normal range (95 mmol/mol creatinine, controls: 10.3-98.8), while creatine excretion was as high as 3586 mmol/mol creatinine (controls: 6-1208), suggesting a creatine transporter deficiency. The creatine uptake profile (cultured fibroblasts) was significantly different from what found in controls. DNA sequence analysis of the SLC6A8 gene demonstrated a hemizygous mutation in the X-linked creatine transporter gene, consisting of A-G transition in intron 1 (IVS1-2A>G). The patient's mother's DNA was assayed and the same mutation was found (G. Salomons, Amsterdam Free University). Creatine transporter deficiency is an X-linked recessive disorder characterized by mental retardation and severe developmental language impairment. A defect in the X-linked creatine transporter SLC6A8 gene, mapped at Xq28 (OMIM 300352) was identified in 2001. Since then, 7 unrelated families (13 male patients and 13 carriers) with an SLC6A8 deficiency have been reported. Herein, we report an additional patient with creatine transporter deficiency caused by a previously unreported SLC6A8 gene mutation.

Progressive obesity in a patient with fumarase deficiency. *P. Campeau*^{1, 4}, *R. Laframboise*¹, *Y. Giguère*², *B. Piedboeuf*³. 1) Medical Genetics; 2) Medical Biochemistry; 3) Neonatology, CHUL and Laval University, Québec, Qc, Canada; 4) Department of Medical Genetics, McGill University, Montréal, Qc, Canada.

The male proband is the child of a non-consanguineous french-canadian couple with a non-contributory family history. Rapidly after birth the child presented metabolic acidosis, hyperlactacidemia at 16 mmol/l and hyperammonemia at 263 mol/l. He was treated for less than 24 hours with bicarbonates and sodium benzoate and his clinical condition rapidly improved. The child had transitory DIC, renal failure, hepatocellular lysis and seizures. Brain MRI showed cortical and subcortical atrophy, 4 cysts on the lateral sides of the frontal ventricular horns, and a right temporal hygroma. Echocardiography showed atrial septal defect. Urinary organic acids showed constant and significant fumaric acid increase. Amino acids and karyotype were normal as well as PDH complex activity on skin fibroblasts. Fumarase activity in skin fibroblasts was significantly decreased in the cytosolic and mitochondrial fractions, confirming fumarase deficiency (mutations pending to correlate genotype with phenotype). The child was treated with a low protein diet, phenobarbital, lamotrigine, L-carnitine, biotin, thiamine, riboflavin, pyridoxine and ranitidine. He experienced progressive clinical deterioration with gastroesophageal reflux, scoliosis, diastolic dysfunction with ventricular hypertrophy, severe hypotonia and developmental delay. He had frequent infections with secondary neutropenia; immunoglobulin and complement studies were normal. Progressive obesity developed despite a tightly controlled caloric intake. Investigations revealed a normal insulin response, TSH, ACTH, cortisol and lipid profile. The child died from complications of an influenza infection at 7 months. This is the first report of progressive obesity in a child with fumarase deficiency. We hypothesize that an increase in mitochondrial citrate (caused by a block in fumarate conversion and oxaloacetate replenishing by anaplerotic pathways) led to subsequent increases in cytosolic acetyl-CoA and in the synthesis of fatty acids.

Case report of a Long-chain 3 hydroxyacyl-coenzyme A dehydrogenase (LCHAD) deficiency presenting with metabolic acidosis and ketosis. *A. Feigenbaum¹, G. Maegawa¹, S. Olpin², A. Strauss³, J. Callahan¹, N. Poplawski¹.* 1) Clinical and Metabolic Genetics, Metabolic Lab, Hosp Sick Children, Toronto, ON, Canada; 2) Dept. of Clinical Chemistry, Sheffield Children's Hosp, West. Bank, Sheffield, UK; 3) Dept. of Peds and Vanderbilt Children's Hosp, Vanderbilt University, Nashville, TN, USA.

We report a case with a fatty acid oxidation defect (FAOD) who on initial clinical presentation showed ketones. This 7-week old male was born at 35 week gestation who developed sudden onset respiratory distress, gallop rhythm and a 3/6 systolic murmur. Initial labs showed a severe metabolic acidosis, high lactate (18; nl 0-2.4), hypoglycemia, ketones in urine and with normal AST, ALT, GGT, ALP. Echocardiogram confirmed severe dilated cardiomyopathy with a reduced ventricular function. Org. acid (GC-MS) showed very large lactic acid, moderate 2-OH-butyric and 3-OH-butyric acids and trace of acetoacetic acid. Plasma total carnitine was 13.8 (32-84), free carnitine 9.7 (26-60) and Acylcarnitine profile: reported increased C14, C16 and C18 acylcarnitines suggestive of LCHAD deficiency. The fatty acid oxidation flux in fibroblasts revealed significantly reduced activity for both enzymes indicating MTP deficiency. Molecular analysis showed he was compound heter. for 2 mutations in the LCHAD/trifunc. prot. alpha-subunit gene: G2027T in exon 19 leading to a substitution of arginine to lysine. An unreported intronic mutation T+30 was found after exon 6. The Western blot analysis using specific antibodies to both the LCHAD and thiolase revealed markedly low antigen levels, less than 2% of control. He had a persistent hypocalcemia and was diagnosed with hypoparathyroidism needing suppl. calcium and calcitriol. He is on long chain fat restricted diet, carnitine supplementation and has responded well. His cardiac function has normalized in about 3 months and remains stable since. The clin-lab presentation of this case was typical of FAOD however the urinalysis and did show ketones. This is an example of hypoketosis rather than nonketotic hypoglycemia as a presentation of FAOD and if present can mislead the work-up, diagnosis and even acute management.

Molecular and functional studies of OCTN2 (SLC22A) in three patients with mild systemic carnitine deficiency.

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Carnitine serves several physiologic functions that regulate organic acid metabolism and ameliorate organic acid accumulation in disease states. These include mediation of transport of long chain fatty acids across the inner mitochondrial membrane for β -oxidation, and esterification of potentially toxic acyl-CoA metabolites that accumulate proximal to a metabolic block. Carnitine transport into cells is mediated by a high affinity transport protein (OCTN2) encoded by SLC22A5, which is one of a family of organic ion transporters. We recently studied three families with clinical and chemical features consistent with mild systemic carnitine deficiency, and found both synonymous and non-synonymous amino acid substitutions, and mutations in the 3' untranslated region, in SLC22A5 in these patients. Transformed lymphoblasts from all three patients showed reduced carnitine transport activity, to approximately 25% (20.5% to 29.6% of control, $p < 0.05$) and reduced levels of OCTN2 mRNA (10.2% to 16.4% of control, $p < 0.05$). The reduction in activity (and mRNA expression level) of OCTN2 is moderate in comparison to that observed in patients with null mutations in SLC22A5 and is consistent with the patients mild clinical presentation. It is possible that the genetic variants of OCTN2 identified in these patients may lead to the quantitative deficiency in the expression and activity of OCTN2. Studies are underway to identify the genetic determinant(s) of the carnitine deficiency observed in the three patients, which include examining the functional effects of polymorphic mutations in OCTN2 and the role of modifier genes and other multifunctional organic cation transporters on carnitine transport in patients with mild systemic carnitine deficiency. The finding that moderate but symptomatic deficiencies in systemic carnitine levels are associated with a moderate reduction in OCTN2 expression simultaneously represents a previously unrecognized clinical syndrome in pediatric patients and suggests its mechanism.

Acute rhabdomyolysis and hepatic failure: a manifestation of respiratory chain deficiency? *M.T. Geraghty¹, J. Michaud¹, B. Robinson², D. Millington³, N. Lepage¹, P. Chakraborty¹*. 1) CHEO, Ottawa, ON; 2) Toronto Sick Kids, ON; 3) Duke University, NC.

A 4 month old female presented with seizures 1 day after immunizations. She had normal growth and development and no contributory family history. On admission she had a metabolic acidosis with hypoglycemia, hyperammonemia and increased lactate. Blood gases showed pH 7.17, pCO₂ 52.9 torr and HCO₃ 19 mmol/L. Chemistry results were Na 141mmol/L, K 6.5 mmol/L, Cl 104 mmol/L, glucose <0.6mmol/L, NH₄ 741 umol/L and lactic acid 10.5 mmol/L. Urine was negative for ketones. She had biochemical evidence of liver and muscle dysfunction. Over the next 48 hours lactate returned to normal. CK levels peaked at 48 hours (36162 U/L) with severe myoglobinuria. Liver function tests worsened through 48-72 hours; albumin 22 G/L, ALT 8000 U/L with increased PT and PTT. Free carnitine was 39 umol/L and total was 102 umol/L. Acylcarnitine profile was suggestive of a multiple acyl-dehydrogenase defect. Urine organic acids showed lactic and dicarboxylic aciduria only. Plasma amino acids were non specific, with increased ala and gln (1679 umol/L and 1076 umol/L). There were moderate elevations in phe, tyr, met, orn, lys and pro. The child responded to intubation and supportive therapy. Biochemistry normalized over the following seven days. In vitro studies of fatty acid oxidation in fibroblasts were normal. A muscle biopsy taken 6 months after discharge showed enhancement of Gomori trichrome and SDH staining with no ragged red fibres. On electron microscopy there was a normal number mitochondria but these were enlarged with increased and irregular cristae and located in the subsarcolemmal space. Analysis of frozen muscle showed low complex I and III activity (0.49 umol/min/g wet weight; normal 0.5-1.9). The ratio to citrate synthase activity was reduced. Studies in fibroblasts had similar but less striking results. Rhabdomyolysis and hepatic failure have been reported in fatty acid oxidation defects but they have rarely been ascribed to mitochondrial defects. While not conclusive the clinical findings suggest that this child's presentation was due to a respiratory chain defect.

Newborn screening for inborn errors of metabolism by tandem mass spectrometry in China. *X.F. Gu^{1,2}, L.S. Han¹, X.L. Gao¹, J. Ye^{1,2}, W.J. Qiu^{1,2}.* 1) Department of Pediatric Endocrinology and Genetic Metabolism, Xin Hua Hosp, Shanghai Second Medical University, Shanghai, China; 2) Shanghai Institute for Pediatric Research, Shanghai 200092, China.

Objective: The aims of this pilot study were to establish an analysis method of amino acid and acylcarnitine profiles on dry blood filter paper using MS/MS and to screen inborn errors of metabolism in Chinese newborns. **Methods:** From January 2003 to May 2004, 22,700 neonates in Shanghai were tested using dried blood spots at the age of 3 to 10 days. A 3mm disk of dried blood filter paper was punched and extracted with stable isotope labeled internal standards, which involve 12 kinds of amino acids and 8 kinds of acylcarnitine, then derivatized with butanolic-HCL in 65 oven for 15 min. After preparation, the samples were reconstituted with acetonitrile: water 80:20 volume and analyzed by MS/MS (API 2000). The data which were detected by MS/MS were calculated by the software (Chemo View 1.2). The specimens for quality control of Amino acid and acylcarnitine were supplied by CDC of America. The Diagnosis of positive newborns was confirmed by the characteristic clinical symptoms, clinical chemistry tests, loading test, and gas chromatography-mass spectrography (GC-MS) analysis. **Results:** Sixty-six newborns (0.28%) were positive and recalled. Seven of the 66 newborns (1/3243) were confirmed as inborn errors of metabolism, which involved 3 hyperphenylalaninemia (HPA), 2 methylmalonic acidemia (MMA), 2 3-methylcrotonyl-CoA carboxylase defecion (3-MCC). Among these 7 children only 2 MMA newborns had vomiting on clinical. Other 5 newborns had no symptoms. The false-positives rate was 0.26% (59/22,700). **Conclusions:** We established a fast, accuracy and sensitive tandem mass spectrometry method for amino acid and acylcarnitine profiles analysis, more than 30 metabolic diseases including amino acid disorders, organic acid disorders and fatty acid oxidation disorders could be detected. The pilot study shown that the inborn errors of metabolism in newborn were 1:3243 in this study, and all cases detected should be long-term surveillance.

A pilot study of selective screening in high risk children with inborn error of metabolism using tandem mass spectrometry in China. *L.S. Han^{1,2}, J. Ye^{1,2}, W.J. Qiu^{1,2}, X.F. Gu^{1,2}.* 1) pediatric endocrinology, xinhua hospital, shanghai, shanghai, China; 2) Shanghai Institute for Pediatric Research, Shanghai, 200092 China.

The aim of the present study was to establish a dry blood filter paper method for amino acid and of acylcarnitine test using tandem mass spectrometry and application on selective screening in high risk children with inborn error of metabolism. Eight hundred high risk cases with inborn error of metabolism were studied, the samples were from in more than 30 hospitals of all over China in November 2002 to May 2004, there were 520 males and 280 females, the means age was 3.46 years. The patient had at least one of the following symptoms: mental retardation, slowly development, psychology abnormal, muscle hypotonia, jaundice, hepatomegaly, recovery vomiting or convulsion, hypoglycemia, hyperammonemia and lactic acidemia. The blood was collected on filter paper and analyzed by tandem mass spectrometry. The diagnosis was confirmed by characteristic clinical symptoms, clinical chemistry tests or gas chromatography-mass spectrography (GC-MS) in part of cases. Ninety-three patients were positive in our selective screening program, including 55 with amino acid disorders(41 hyperphenylalaninemia, 3 tyrosinemia, 1 homocystinuria, 1 hypermethioninemia, 2 citrulinemia, 2 maple syrup urine diseases, and 5 ornithine transcarbamylase deficiency), 32 with organic acid disorders(16 methylmalonic acidemia, 4 propionic acidemia, 2 isovaleric acidemia, 4 glutaric acidemia, 2 3-methylcrotonyl CoA carboxylase deficiency, 1 3-hydroxy-3-methylglutaryl-CoA lyase deficiency, 2 biotinidase deficiency, and 1 b-ketothiolase deficiency), and 6 with fatty acid oxidation disorders (2 medium chain acyl-CoA dehydrogenase deficiency, 2 carnitine palmitoyl transferase type II, and 2 multiple acyl-CoA dehydrogenase deficiency). Our results indicated the importance of selective screening on high risk patients of amino acid disorders, organic acid disorders and fatty acid oxidation disorders with tandem mass screening. The most of diseases that cause of death and disability represent a preventable by early diagnosis and treatment.

Molecular Basis for Glucose-Galactose Malabsorption In Large Kindred. *D. Kesselman*^{1, 3}, *O. Reish*², *N. Gal*³, *H. Gore*³, *Y. Bujanover*³, *Y. Anikster*³. 1) Bio Resources Engineering, University of Maryland, College Park, MD; 2) Dept Genetics, Asaf Harofe Hospital; 3) Metabolic Disease unit, Safra Children's Hospital, Tel Hashomer.

Glucose Galactose malabsorption (GGM) is a rare autosomal recessive disorder characterized by life threatening diarrhea at infancy. When the intake of the offending sugars is ceased, the diarrhea promptly stops. The diet given to patients with GGM is free of glucose and galactose and supplemented with fructose. A Mutation in the *SGLT1*- the sodium-glucose co-transporter gene, causes a defect in the glucose-galactose transport across the intestinal lining which is responsible for the disease.

We examined three children from two families of Israeli Arab Muslim origin who have a history of GGM and receive the appropriate diet. We performed the breath hydrogen test with D-Glucose and lactose which confirmed the diagnosis for one of the patients. Peripheral blood samples were taken from the patients, their siblings and their parents and DNA was extracted. Genomic DNA of a patient was PCR amplified for each of the 15 exons of the *SGLT1* gene and analyzed by direct nucleotide sequencing.

We identified a C to G substitution at nucleotide 765. This caused a novel missense mutation C255W. Even though this novel mutation is not on one of the 14 predicted transmembrane domains of the SGLT1 protein, the change from sulfur containing cysteine to the aromatic tryptophan along with the segregation in the kindred further support that this is a pathological mutation. This mutation created an *RsaI* restriction site and using the exon 8 amplicon we showed full segregation in these large kindred. Our results allow for prenatal diagnosis and carrier detection in this unique genetic isolate.

Polymorphism analysis of ATP7B related microsatellite DNA haplotype in Wilson disease. X. Liu^{1,2}, Y.F. Zhang^{1,2}, L. Wang¹, X.F. Gu^{1,2}, K.R. Bao¹. 1) Pediatric, Xinhua Hospital, Shanghai, Shanghai, China; 2) Shanghai Institute for Pediatric Research, Shanghai 200092, China.

Objective To explore the distribution and significance of haplotype of microsatellite DNA(D13S314,D13S301 and D13S316) closely related to ATP7B in normal population , WD patients and heterozygotes. **Methods** Using three well characterized short tandem repeat markers of fluorescence labelling (D13S301-D13S314-D13S316), localization and sequence were analysed with Genotype™ software in 71 WD patients and 123 parents. **Results** Based on the analysis of haplotypes of D13 S314,D13S301 and D13S316, 19, 20, and 15 alleles were obtained in 71 patients with WD, 123 carriers and 54 normal persons respectively, size of segments was 134-157bp, 128-156 bp, and 136-154 bp. Heterozygosity was 0.79, 0.82, and 0.23. There was significant difference in distribution of alleles of D13S314,D13S301 and D13S316 makers between WD patients and normal person($P < 0.01$), 4 alleles in D13 S314 maker, 6 alleles in D13 S316 maker. There was 81 haplotypes. 12-6-5, 15-10-5, 6-10-5 and 6-15-5, the commonest haplotypes, accounted for 5.22%, 4.48%, 4.48% and 3.37% respectively. 12-8-5, 12-7-5 and 6-16-5 separately accounted for 2.99%, 13-10-8, 6-13-5, 6-14-13 and 6-9-5 separately accounted for 2.24%. **Conclusion** The complexity of haplotype may be related to the complexity of mutation spectrum of ATP7B. The haplotype analysis of D13 S314-D13S301-D13S316 is one of effective and feasible method, having important significance, in presymptom and prenatal diagnosis of Wilson disease.

Modeling pathogenesis of human glycerol kinase deficiency (GKD) in glycerol kinase (Gyk) knockout mice. *N.K. MacLennan¹, N. Kuwada¹, Y.H. Zhang¹, Z. Fang^{2,3}, S. Horvath^{2,3}, L. Rahib⁴, E.R.B. McCabe^{1,2}, K.M. Dipple^{1,2}.* 1) Pediatrics; 2) Human Genetics; 3) Biostatistics; 4) School of Engineering, UCLA, Los Angeles, CA.

GKD is an X-linked inborn error of metabolism with episodes of metabolic acidosis. We hypothesized that Gyk knockout (KO) mice would be a model for GKD pathogenesis and tested this by biochemical, microarray and Pathway Assist analysis of Gyk KO and wildtype (WT) mice. We confirmed that Gyk KO mice had hyperglycerolemia, elevated plasma free fatty acids and die by day of life (dol) 3-4 (Huq et al Hum Mol Genet, 1997). We determined (iSTAT Portable Clinical Analyzer) KO male mice were acidemic (pH 7.1), had low plasma HCO₃ (15), and were hypoglycemic (30) on dol 3 compared to WT mice (p<0.05). To understand the molecular mechanism of GKD, we performed microarray analyses on liver mRNA (Affymetrix Mouse 430 Gene Chips). Gene filtering identified 237 differentially expressed (>2-fold) genes involved in metabolism, transcription factor activity, signal transduction, transport, DNA binding, protein modification, cell death and cytoskeleton. Real time PCR confirmed differences in Gyk (12% of WT), glycerol phosphate dehydrogenase 2 (35% of WT), and PPAR gamma coactivating factor 1 (234% of WT). EASE analysis identified 56 increased (p<0.01) and 37 decreased (p<0.01) gene categories in Gyk KO mice. Expression levels of members of gene functional groups involved in organic acid metabolism/transport, lipid metabolism, fatty acid/long-chain fatty acid metabolism, and glucose metabolism were increased (p<0.001). Glycerol/glycerol ether metabolism and steroid biosynthesis gene functional group members were decreased (p<0.001). Pathway Assist (Stratagene) analysis confirmed that perturbations of GK affects pathways involved in organic acid metabolism indicating they are part of the same metabolic network. In conclusion, Gyk KO hepatic mRNA levels are altered in a broad range of functional categories, some of which are relevant to the biochemical aberrations in GKD. Investigations of altered gene expression and metabolite flux in the this GKD murine model will provide valuable insights into GKD pathogenesis.

Cholesterol synthesis and brain cholesterol turnover in PKU. *L.S. Merkens¹, W.E. Connor², M. Gillingham¹, C.O. Harding^{1,4}, D. Lutjohann³, K. von Bergmann³, R.D. Steiner^{1,4}.* 1) Dept Pediatrics, Oregon Health & Science Univ, Portland, OR; 2) Dept Medicine, Oregon Health & Science Univ, Portland, OR; 3) Dept Clin Pharm, Rheinische Fredrich-Wilhelms Universitat, Bonn, Germany; 4) Dept Mole & Med Genetics, Oregon Health & Science Univ, Portland, OR.

Phenylketonuria (PKU) is an inborn error of metabolism caused by phenylalanine (Phe)hydroxylase deficiency causing high blood Phe concentrations. PKU treatment is based on a diet restricted in animal protein and, therefore, cholesterol. Phe inhibits HMG CoA reductase (HMGCR). We hypothesized whole body cholesterol synthesis would be higher and brain cholesterol synthesis lower in PKU vs. controls. HMGCR inhibition by phe would be overridden by the low cholesterol diet systemically, but brain would bear the full effect of HMGCR inhibition since dietary cholesterol doesn't reach the brain. Ten children with PKU were compared to 8 controls. Mean plasma Phe was 6 mg/dL at the time of study, indicating good diet adherence. Plasma cholesterol was measured by gas chromatography (GC); plasma lathosterol and 24S-hydroxycholesterol(24S) by GC/MS. Mean plasma cholesterol concentration was significantly lower in PKU vs controls (132 vs 169 mg/dL). Plasma lathosterol/cholesterol ratio, an estimate of total cholesterol synthesis, was increased in PKU. Plasma 24S, an estimate of brain cholesterol turnover did not differ significantly between groups (195 vs 213 ng/dL). Control children showed a trend of higher 24S at younger ages (linear regression $P=.07$). In PKU, 24S showed no change from 4 mo to 4 yr. In conclusion, low plasma cholesterol levels are consistent with lower dietary cholesterol intake in PKU. Whole body cholesterol synthesis is increased in PKU likely due to lower dietary cholesterol intake. Finally, 24S was the same in PKU and controls, but may be lower in younger PKU children. This finding suggests that Phe may inhibit brain cholesterol synthesis during the period of rapid myelination. Reduced brain cholesterol synthesis could play a role in executive function deficits and hypomyelination observed in PKU.

The frequency of sudden early neonatal death (SEND) and fatty acid oxidation disorders in New South Wales, Australia. *J. Mitchell*^{1, 2}, *V. Wiley*¹, *K. Sim*¹, *K. Carpenter*¹, *B. Wilcken*¹. 1) The Children's Hospital at Westmead, Sydney, NSW, Australia; 2) Montreal Children's Hospital, Montreal, Quebec, Canada.

Purpose: Sudden early neonatal death after uncomplicated pregnancy is a rarity. The differential diagnosis includes cardiac causes, trauma, infection and metabolic disease. Of the metabolic disorders, fatty acid oxidation defects (FAOD) are the most likely to present in this way. Among a birth cohort of 545,000, we describe four cases of sudden early neonatal death occurring in the first three days of life, secondary to different fatty acid oxidation disorders.

Methods: Screening for abnormal metabolites in all suspect cases was performed by tandem mass spectrometry using samples obtained for newborn screening. Amongst four neonatal deaths, the dried blood spots from two were taken post-mortem, and the indicative abnormal profiles were confirmed by mutation testing.

Results: The diagnoses were: medium-chain acyl-CoA dehydrogenase deficiency, multiple acyl-CoA dehydrogenase deficiency (glutaric aciduria Type II), and two cases of acylcarnitine-carnitine translocase deficiency. At autopsy, all four had hepatic steatosis. These babies were among a birth cohort screened by tandem mass spectrometry over a six year period (1998-2004). In that cohort we diagnosed a total of 43 cases of FAOD, amongst whom these 4 (9%) suffered neonatal death. We estimate a minimum of 1:60 term neonatal deaths in New South Wales are metabolic SEND, and if only unexpected deaths in the first week were considered this would be much higher.

Conclusions: Although the diagnosis did not alter clinical outcome in these neonates, it did allow for genetic testing in family members and future pregnancies. These cases illustrate the importance of obtaining samples post-mortem on all babies dying unexpectedly and the value of dried blood spots and tandem mass spectrometry in the detection of FAOD. Neonatal death amongst FAOD patients seems more common than previously thought, and FAOD are an important cause of unexpected early neonatal death.

Detection of mutations Q188R, K285N and S135L in GALT gene in Iranian galactosemia patients. N.

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100 unrelated families, clinically suspected to galactosemia, were screened by qualitative measurement of galactose-1-phosphate uridyl transferase (GALT) activity in blood RBCs using Beutler method. Deficient enzyme activity (classical galactosemia) was confirmed in 13 families. All of these 13 families were submitted to the diagnosis of six common mutations in GALT gene including Q188R, K285N, S135L, L195p, X380R and Q169K using PCR-RFLP method which resulted in detection of 46% of the mutated alleles. 6 patients were homozygote for Q188R mutation, while one patient homozygote for S135L mutation and one heterozygote for K285N mutation were detected.

Molecular Characterization of Lesch Nyhan Syndrome in Puerto Rican Family. *P. O'Neill¹, A.S. Cornier², N. Arciniegas³, J. Acevedo⁴, N. Ramirez⁵, S. Carlo².* 1) Genetics Lab, Univ Vermont, Burlington, VT; 2) Genetics Division, Ponce School of Medicine, Ponce, PR; 3) Department of Pediatrics, Ponce School of Medicine, Ponce, PR; 4) Nursing Dept. Univ of Puerto Rico at Arecibo, Arecibo, PR; 5) Orthopedics Dept., Mayaguez Medical Center, Mayaguez, PR.

Lesch-Nyhan (MIM 308000) syndrome is an inherited deficiency of the purine salvage enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT) characterized by neurologic dysfunction, cognitive and behavioral disturbances, and uric acid overproduction (hyperuricemia). The neurologic findings most closely resemble athetoid cerebral palsy. The most common presenting features are hypotonia and developmental delay, which are evident by three to six months of age. Patients are delayed in sitting; most never walk. Within the first few years, extrapyramidal involvement (e.g., dystonia, choreoathetosis, opisthotonus) and pyramidal involvement (spasticity, hyperreflexia and extensor plantar reflexes) become evident. Patients are cognitively impaired and have behavioral disturbances that emerge between two and three years of age. Persistent self-injurious behavior (biting the fingers, hands, lips, and cheeks; banging the head or limbs) is a hallmark of the disease, clinical manifestations are more evident with less than 1.5 percent of residual activity of the enzyme. Enzymatic residual activity between 1.5 and 8 percent produces phenotypes with mild neurological manifestations or hyperuricemia alone. Being an X-linked disorder (Xq26-27.2) females with the disorder are rare, only five reported (Jinnah et al). We studied a family from the central part of Puerto Rico in which there are three affected male individuals, one affected female and two female carriers. Male patients had been evaluated but did not have a definite diagnosis. The affected female was evaluated due to hypotonia and developmental delay. Metabolic work up that included carnitine profile, urine organic acids and lactate and pyruvate was negative. Patient presented elevated plasma uric acid and started with self injury behavior around 18 months of age. HPRT activity was measured and diagnosis was made. Mutation analysis of HPRT gene is currently being performed.

Function of two alternatively spliced mRNAs from the Xp21 glycerol kinase gene. *R. Ohira*¹, *E.R.B. McCabe*^{1,2}. 1) Depts of Hum Genet; 2) Pediatr, David Geffen Sch of Med at UCLA, LA, CA.

Glycerol kinase (GK) is involved in fat and carbohydrate metabolism and is alternatively spliced. GK phosphorylates glycerol to form glycerol 3-phosphate, which is important in production of glycerol lipids and glycerol ether lipids. An 87 bp insertion corresponding to exon 18 of the Xp gene (*GK+EX18*) was isolated from human fetal brain and testes. The predominant form of GK found in liver lacks exon 18. Our goal was to determine differences in kinetics and localization within the cell for GKEX18 (GK- or GK+). For kinetic studies, Cos7 cells were transiently transfected with GK- or GK+, and GK activity assayed using whole cell extracts. K_m and V_{max} were derived by Hanes-Woolf analysis. K_m for glycerol and ATP were 2.2M and 6.4M for GK- and 2.6M and 12.2M for GK+, respectively. V_{max} for glycerol and ATP were 16,540U/mg and 1,914U/mg for GK-, and 11,752U/mg and 3,167U/mg for GK+, respectively. K_m values for glycerol were not significantly different (P0.05), but GK+ had a higher K_m for ATP (P0.05). GK- had a significantly higher V_{max} for glycerol (P0.05), but lower V_{max} for ATP (P0.05). Fractionation showed that GK+ had very little activity in the cytosolic, some activity in the mitochondrial, and highest activity in the nuclear fraction. However, GK- had the highest activity in the cytosol, and some activity in the mitochondrial and nuclear fractions. Immunostaining was done on Cos7 cells transiently transfected with Flag-tagged GKEX18 and various markers to identify organelles for colocalization studies. GK+ colocalized mainly to the mitochondria, whereas GK- had a more diffuse expression pattern, suggesting specific functions for the two forms. GK+ may be involved with mitochondrial specific processes, like regulating energy metabolism or apoptosis. GK- may have a metabolic function in the cytoplasm, but also may play a specific role in the nucleus as the ATP-stimulated glucocorticoid receptor translocation promoter (ASTP). ASTP enhances nuclear uptake and binding of glucocorticoid receptor complexes in the presence of ATP. Additional research will be required to determine whether ASTP activity is associated with a single GK isoform.

Immunodetection and mutational analyses in 34 patients with enzymatically diagnosed pyruvate dehydrogenase complex (PDC) deficiency. *K. Okajima*^{1,2}, *M.L. Warman*², *K.S. Kerr*¹. 1) CIDEM, Rainbow Babies & Children's Hosp, Cleveland, OH; 2) Departments of Pediatrics and Genetics, Case Western Reserve University, Cleveland OH.

PDC deficiency is a common cause of primary lactic acidemia. In many cases, mutations are found in *PDHA1* (E1) which maps to Xp22. We analyzed *PDHA1* mutations and alterations of PDHA1 protein on immunoblot among 34 patients (21 male, 13 female) who had been diagnosed by enzyme assay. All patients had normal DLAT (E2) and DLD (E3) activity. **Method:** Genomic DNA (or cDNA) was analyzed by PCR (or RT-PCR) and direct sequencing. Immunoblotting of fibroblast lysates from 10 males and 8 females with mutations and 4 males and 4 females without mutations, was performed using an anti-PDHA1 monoclonal antibody and anti-succinate dehydrogenase as a control. **Result:** Mutations were found in 24 of the 34 patients. 15 patients (9 male, 6 female) had point mutations, 7 patients (4 male, 3 female) had small insertions, one male had a small deletion, and one male was a somatic mosaic for a point mutation leading to exon-skipping. Only one point mutation and one insertion were found in multiple families. Among the 10 males with mutations, 6 had normal and 4 had reduced PDHA1 by immunoblot; 4 males without *PDHA1* mutations had normal PDHA1. Among females with mutations, 5 had normal PDHA1 and 2 had reduced amounts of immunodetectable PDHA1. In the latter 2 samples, enzyme activities were very low and cDNA analyses suggested skewed X-inactivation. **Discussion:** In patients at risk for *PDHA1* mutation, we detected mutations in 15 of 21 males and 9 of 13 females. Males without mutations had normal appearing PDHA1 protein by immunoblot, suggesting that hemizygous *PDHA1* mutations were not the cause of their reduced PDC activity. Similar conclusions are difficult to draw in females with reduced PDC activity and an undetectable mutation because large deletions could be missed in heterozygous females using our strategy. Immunoblot analyses are less sensitive than DNA or RNA based methods for finding PDHA1 mutations, since altered protein levels were noted in only 40% of male and 25% of female samples with mutations.

Effect of genetic excess of gamma-hydroxybutyric acid (GHB) and gamma-aminobutyric acid (GABA) brain secretion on sleep. *A. Philippe¹, I. Arnulf², K.M. Gibson³, D. Rabier⁴, P. Beauvais⁵, A. Munnich¹, J.P. Derenne².* 1) INSERM U 393, Hopital Necker-Enfants Malades, Paris, France; 2) Fédération des pathologies du sommeil, Hopital Pitié-Salpêtrière, Paris; 3) Biochemical Genetics Laboratory, Oregon Health Sciences University, Portland; 4) Laboratoire de Biochimie Médicale B, Necker-Enfants Malades, Paris; 5) Service de Neuropédiatrie, Hopital Trousseau, Paris.

Background: Exogenous gamma-hydroxybutyric acid (GHB) increases slow wave sleep in healthy volunteers and reduces daytime sleepiness and cataplexy in patients with primary narcolepsy. Objective: To examine night-time sleep and daytime vigilance in a 13 yr-old girl homozygous for succinic semialdehyde dehydrogenase (SSADH) deficiency, a rare autosomal recessive metabolic disorder that disrupts the normal degradation of gamma-aminobutyric acid (GABA), and leads to an accumulation of GHB and GABA within the brain. Methods: Sleep interview and unattended polysomnography over 48 hours (habituation night, multiple sleep latency tests, and continuous 24-hours in lab recordings). Her mother heterozygous for SSADH deficiency and a 13 yr-old control girl were also interviewed and recorded during the night. Results: During quiet wakefulness, background EEG activity was slow, composed of 7 Hz activity. Sleep symptoms, duration, and architecture (particularly stages 3-4), were within normal ranges for the patient and her mother, but daytime mean sleep latency was short (3 min 42 s) in the patient while spindle indexes were low in the child (11/hr) and in the mother (39/h), compared to 275/h in the control. At the beginning of the second night, a tonic-clonic seizure occurred, followed by a dramatic increase in stages 3-4, representing 338 min or 56 % of the time asleep. Discussion: Our observations suggest that regular GHB and GABA excess causes subtle sleep abnormalities while a sudden event (here an epileptic seizure) possibly responsible for an extra-increase in GABA and GHB, results in increased slow wave sleep.

Transcobalamin II deficiency: an under recognized cause for bone marrow failure in childhood. *C. Prasad¹, K. Corley¹, A.E. Cairney¹, C.A. Rupa^{1,2}*. 1) Department of Pediatrics, CHWO &; 2) Biochemistry and Pathology, CPRI, London, ON, Canada.

We report on 3 affected sibs (Albanian) with Transcobalamin II (TC II) deficiency. The proband presented at 3 months of age with pancytopenia, failure to thrive, and suspected Fanconi anemia. Intensive investigations, and aggressive management with granulocyte colony stimulating factor, erythropoietin, total parenteral nutrition, gastrostomy feeding, repeated platelet and blood transfusions were unrewarding. Bone marrow transplant was being considered when methylmalonic aciduria was detected with no specific abnormalities on plasma and urine amino acids. The plasma B12 levels were normal. There was a previous sibling death due to pancytopenia. Further confirmatory testing on the proband revealed very little synthesis of TC II by cultured fibroblasts in the absence of exogenous TC II (Dr. David Rosenblatt, McGill University). There was adequate synthesis of AdoCbl and MeCbl. A novel mutation homozygous R399X C>T substitution at nt 1195 of cDNA resulted in a premature stop codon. The proband responded very well to Vitamin B12 (~2.5 mg) with complete resolution of symptoms and normalization of growth and development. A third child (girl) was treated with Vitamin B12 from the age of 3 weeks and confirmed by mutation analysis to also have TC II deficiency. She is doing well with Vitamin B12 replacement, which will be continued, for life as for her brother. TC II deficiency is an autosomal recessive disorder. TC II, a plasma globulin is necessary for delivery of cobalamin molecule to the hematopoietic system. The vitamin B12 levels are usually normal. TC II is a member of cobalamin binding proteins in humans that include intrinsic factor, transcobalamin I and a putative protein. The TC II gene is a housekeeping gene, which has a unique 34-nucleotide stretch sequence that is a motif for a transactive transcription factor that regulates expression of epidermal growth factor receptor gene. The availability of an extremely effective B12 treatment should prompt the physicians to consider this diagnosis earlier in the work up of bone marrow failure syndromes in childhood.

Prenatal diagnosis of glycogen storage disease Ia with hot spot mutations screening and 1176 nucleotide polymorphism linkage analysis. *W. Qiu^{1,2}, J. Ye^{1,2}, L.S. Han^{1,2}, X.F. Gu^{1,2}.* 1) Pediatric Endocrinology, Xinhua Hospital, Shanghai, Shanghai, China; 2) Shanghai Institute for Pediatric Research, Shanghai 200092, China.

Objective This study was to obtain a simple, fast and accurate prenatal diagnosis method for glycogen storage disease Ia (GSD Ia). **Methods** Genomic DNA samples were abstracted from the bloods of 2 GSD Ia patients from 2 families, their parents, uncultured and cultured amniocytes of the 2 fetuses. Screening for the 727GT and R83H mutations of glucose 6 phosphatase combined with 1176 nucleotide polymorphism linkage analysis by restriction enzyme analysis were used to make the genetic and prenatal diagnosis of 2 GSD Ia families. Direct DNA sequencing of the corresponding PCR products were used to confirm the found mutations and 1176 nucleotide polymorphism. **Results** The 2 probands of these 2 families were homozygous for the 727GT mutation and their parents were heterozygous for this mutation. The fetus of X family was heterozygous for this mutation and the fetus of L family did not carry this mutation. The 1176 nucleotide polymorphism of the 2 fetuses was different from those of the probands of the 2 families. The prenatal diagnosis of the X family was confirmed postnatally by the biochemical and molecular studies. **Conclusion** Our finding suggest that the screening for the 727GT and R83H mutations by restriction enzyme analysis combined with 1176 polymorphism linkage analysis is a simple, fast and accurate method for gene and prenatal diagnosis for GSD Ia in Chines.

Classic and Duarte Galactosemia in Louisiana: Newborn Screening Detection and Treatment Outcome. R.

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Galactosemia newborn screening was done in 121,258 infants in Louisiana during June 2002 May 2004 by detecting semiquantitative galactosemia -1-phosphate uridylyltransferase (GALT). A GALT value $<3.4\text{U/gHb}$ was defined as presumptive positive galactosemia and was confirmed by measuring quantitative GALT. GALT $<10\text{mol/g Hb}$ (50% of low normal) was further tested by isozyme or mutation analysis to classify the type of affected galactosemia (D/D, D/G, G/G). There were 64 presumptive positive cases of galactosemia. After confirmatory tests, 8 cases were normal, 30 cases were galactosemia carriers and 24 were affected with galactosemia (2 G/G, 3D/D, 11D/G and 8 no information). The incidence of galactosemia in Louisiana is 1: 4,826 livebirths (including Duarte variant) and 1: 57,920 (not including Duarte variant) compared to the national incidence rate of 1:53,261. All except one case of classic galactosemia were healthy at the time of diagnosis. Some patients had mild GI symptoms such as reflux, constipation, vomiting etc. during lactose containing diet. All affected galactosemia patients, including Duarte variant galactosemia (D/G, D/D) patients were treated with lactose-free diet for at least 1 year. Duarte variant galactosemia patients (D/G, D/D) were challenged with lactose diet protocol by age 1 year. 7 Duarte variant patients were challenged with lactose diet by age 1 year and all passed the test (Galactose-1-phosphate or Gal-1-p $<2\text{mg}/100\text{ml RBC}$). The average Gal-1-p during restriction and after challenge test in these 7 patients was 0.57 and 1.01mg/100ml RBC, respectively ($p=0.08$). 2/7 Duarte patients were challenged at 2 months old because of parental concern; one failed the test (gal-1-p was 12.7 after challenge) and the other one passed and was given unrestricted diet. Treatment of Duarte variant galactosemia from newborn screening has not been standardized. Our preliminary data do not demonstrate pathology in Duarte variant patients. Follow up is needed to determine whether Duarte variant patients develop cataracts or other late sequelae of galactosemia.

High carrier rate for cystinosis in the Ontario Old Order Amish. V. Siu^{1,2,3}, S. Kuepfer³, R. Dewar², C.A. Rupa^{1,2,3}. 1) Dept Pediatrics and Biochemistry, Univ of Western Ontario; 2) Child and Parent Resource Institute; 3) London Health Sciences Centre, London, ON, Canada.

The Old Order Amish (OOA) in Ontario represent a highly consanguineous and conservative population with a genetic background which differs significantly from that of the OOA in Lancaster County, Pennsylvania. In 1825, the founding members began immigrating directly to Ontario from Alsace-Lorraine. Since then, there has been minimal influx of new genes. Currently, there are about 220 married couples in the area, representing a population of about 900. There are only 9 surnames, 8 of which were present in 1859. About half of the families share the same surname, and 25% of marriages are isonymous. Genetic disorders which are common in Lancaster are notably absent in the main OOA settlements in Ontario, while other disorders not seen in Lancaster have been repeatedly observed in Ontario. The mutations causing 3 treatable disorders, cystinosis, cystic fibrosis, and juvenile-onset glaucoma, in Ontario OOA have been identified. Consultation with Amish bishops established that prenatal screening would not be an option, but that newborn screening for presymptomatic diagnosis and treatment would be acceptable. A newborn screening project was initiated. DNA obtained from umbilical cord samples is analysed for the 4 mutations known to be present in the community. Although carrier status is ascertained, this information will not be revealed until the child has reached adulthood. Thus far, 36 babies have been screened, representing about 80% of all newborns, with a distribution of surnames similar to the entire community. No infant was predicted to be affected. None of the couples had a previously affected child. A high carrier rate of 1:5 for cystinosis was found, predicting an incidence of 1:100 for cystinosis. Two newborns were double heterozygotes, one for cystinosis and glaucoma and one for cystinosis and cystic fibrosis. Carrier rate for cystic fibrosis was 1:9 and for glaucoma was 1:18. The extremely high carrier rate for cystinosis suggests that the mutation was present in a founder and has become prevalent due to continued inbreeding and random drift.

Decreased glycogen content in galactose-1-phosphate uridylyltransferase(GALT) -deficient patient cells. *T. Slepak¹, N. Longo², K. Lai¹.* 1) Dept Pediatrics, Univ Miami, Miami, FL; 2) Dept Pediatrics, Univ Utah, Salt Lake City, UT.

In humans, deficiency of GALT produces the disorder Classic Galactosemia. Although newborn screening & a galactose-restricted diet prevent the acute toxicity associated with this genetic disease, long-term complications such as ataxia, verbal dyspraxia and premature ovarian failure persist in some well-treated patients. The pathogenic mechanisms producing dysfunction of multiple organs are unknown. Previously we demonstrated reduced UDP-glucose and some glycoproteins in galactosemic patient fibroblasts (Lai, et al., 2003, Slepak & Lai, 2003).

In animal cells, glycogen is an important carbohydrate reserve that protects the cells against stress factors. Since UDP-glucose is required for glycogen synthesis, we hypothesized reduced UDP-glucose in GALT deficiency impairs glycogen synthesis. We compared the steady state level of glycogen in fibroblasts derived from normal and galactosemic patients. We found 33% or higher glycogen contents in the controls. We, however, did not see a significant difference in the steady state level of ATP in the patient cells. The rate of glycogen depletion also appeared to be the same when these cells were transferred to hexose-free medium. This suggested that the lower glycogen content in galactosemic cells was not resulted from increased consumption, but from reduced synthesis or accumulation.

To determine if decreased uptake of glucose in the galactosemic cells caused the decreased glycogen synthesis, we assayed 3-o-methyl-D-glucose transport in these cells, but found no difference. We later confirmed that the galactosemic cells accumulated 0.3mM galactose-1-phosphate in medium without galactose. The level of UDP-glucose was twice as high in the controls. We concluded that reduced UDP-glucose under GALT deficiency led to decrease glycogen synthesis. As the galactosemic cells perished faster than controls when transferred to hexose-free medium, we propose that the diminished glycogen contents in GALT deficiency may compromise the viability of other cell types under stress conditions.

Haplotype analysis of the region surrounding *CPT1A* in three unrelated Aboriginal kindreds with CPT1 deficiency. A.M. Stier¹, R.J.A. Wanders², L. IJlst², A. Chan³, F. Bamforth³, P. MacLeod⁴, R.J. Thompson⁵, L.E. Seargeant¹, C.R. Greenberg¹. 1) Department of Biochemistry & Medical Genetics, University of Manitoba, Winnipeg, Canada; 2) University of Amsterdam, The Netherlands; 3) University of Alberta, Edmonton, Canada; 4) University of Victoria, Victoria, Canada; 5) Cadham Provincial Laboratory, Winnipeg, Canada.

Carnitine Palmitoyltransferase (CPT) 1 and 2 facilitate the transfer of long chain fatty acids across the outer and inner mitochondrial membranes respectively. A deficiency in the activity of CPT1 may result in hypoglycemia and organ steatosis. A premature Inuit neonate whose mother had AFLP was found to have absent CPT1A enzyme activity and to be homozygous for the CPT1A P479L mutation. CPT2 activity was normal and the child was diagnosed with CPT1A deficiency. The second and third children in this family have had hypoketotic hypoglycemia, are homozygous for the CPT1A P479L mutation and have near zero CPT1A activity. They are also homozygous for a mutation in CPT2 (F352C). A fourth child is also homozygous for both mutations with no discernible phenotype at this time. Fourteen of the 19 extended family members studied were also found to be homozygous for the CPT1 P479L mutation but have never had documented hypoglycemia. Two other symptomatic individuals, a child from an unrelated Inuit family and a First Nations adult male, have also been found to be homozygous for the CPT1A P479L mutation. The sibs and parents in the second symptomatic Inuit family are also all homozygous for CPT1A P479L and asymptomatic. No one in this kindred, nor the First Nations male, are homozygous for CPT2 F352C; therefore this mutation does not appear to be a major contributing factor to the phenotype. Symptomatic individuals in the 3 kindreds were discordant for haplotypes generated by microsatellite genotyping of the region surrounding *CPT1A*, suggesting that the CPT1A P479L mutation either arose more than once or is a very ancient mutation. Whether the *CPT1A* mutation is a benign variant, the true causative mutation or a major factor in the expression of CPT1A deficiency is being investigated in additional prospective and retrospective studies.

Mutations in *OCRL1* occur in patients with renal Fanconi syndrome without congenital cataracts. *S.F. Suchy*¹, *J. Snoops*¹, *R.L. Nussbaum*¹, *R.R. Hoopes, Jr.*², *A. Shrimpton*², *S.J. Knohl*², *P. Hueber*², *B. Hoppe*³, *J. Matyus*⁴, *A. Simckes*⁵, *V. Tasik*⁶, *B. Toenshoff*⁷, *S.J. Scheinman*². 1) Genetic Disease Res Branch, NHGRI/NIH, Bethesda, MD; 2) Dept. of Medicine, SUNY Upstate Medical University, Syracuse, NY; 3) University of Cologne, Germany; 4) University of Debrecen Medical School, Debrecen, Hungary; 5) Children's Mercy Hospital, Kansas City, MO; 6) Clinic for Children's Diseases, Skopje, Macedonia; 7) University Children's Hospital, Heidelberg, Germany.

Lowe syndrome (OCRL) is a rare X-linked disorder characterized by bilateral congenital cataracts, renal Fanconi syndrome and mental retardation. While the renal phenotype can vary in onset and severity, and the diagnosis is compatible with a normal I.Q., bilateral congenital cataracts are considered the hallmark of the disease and the only universal feature. In a study of Dent disease (DD), an X-linked renal tubular disorder associated with mutations in the chloride channel gene, *CLCN5*, 13 of 32 probands meeting strict phenotypic criteria for DD were identified without mutations in *CLCN5*. Linkage in one family to Xq26.3 suggested *OCRL1* as a candidate. Five distinct, previously undescribed mutations in *OCRL1* were found among the 13 DD probands without *CLCN5* mutations. Loss of PIP2 phosphatase activity and absent/reduced *ocrl1* protein in fibroblasts by western blot confirmed that these were disease-causing mutations. In one of these 5 families, an *OCRL1* missense mutation segregated with the phenotype through 3 generations, indicating that either the gene that modifies the phenotype is tightly linked to *OCRL1* or that this specific mutation is responsible for the unusually mild phenotype. These data demonstrate that 1) DD is genetically heterogeneous, 2) defects in *OCRL1* may result in greater phenotypic variability than previously known 3) all patients with mutations in *OCRL1* do not have cataracts and 4) other gene(s) may be involved in the expression of the OCRL phenotype. A study of these patients may allow us to identify gene products and compensatory mechanisms for *OCRL1* deficiency and provide clues as to how to improve function without replacing the *ocrl1* protein.

Increased long chain fatty acid synthesis in SCAD deficient mice. *D. Wong, S. Bassilian, L. Boros, W-N.P. Lee.*
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In order to gain a better understanding of the pathophysiology of the fatty acid oxidation disorder in short chain acyl-CoA dehydrogenase (SCAD) deficient mice, we studied de novo lipogenesis in the mutant mouse strain BALB/cByJ using the stable isotope tracer D₂O. Both diseased and control mice were fed a low fat diet with drinking water enriched with 6% D₂O for 10 days. Plasma and liver tissue fatty acids were analyzed using gas chromatography/mass spectrometry. Mass isotopomer distribution analysis was used to differentiate between newly synthesized and preexisting fatty acids. Liver weights were similar in both groups. Levels of long chain fatty acids in liver tissue were significantly elevated in SCAD deficient mice compared to control mice--palmitate, 35.34.8 vs. 17.62.6 mg/g liver (p<.0001), stearate, 7.60.6 vs. 6.30.3 mg/g liver (p<.001), and oleate, 25.94.8 vs. 10.12.4 mg/g liver (p<.0001). Most of the long chain fatty acids were derived from new synthesis, which was significantly elevated in SCAD deficient mice compared to control mice--palmitate, 30.14.0 vs. 14.12.6 mg/g liver (p<.0001), stearate, 6.30.6 vs. 4.90.2 mg/g liver (p<.001), and oleate, 16.53.3 vs. 6.01.9 mg/g liver (p<.0001). Similar statistically significant elevations were seen in the total and newly synthesized amounts of palmitate, stearate, and oleate in plasma. The results support the hypotheses that (1) fatty acid synthesis is increased in SCAD deficiency in order to compensate for the energy deficit caused by the lack of complete beta oxidation of long chain fatty acids and (2) increased fatty acid synthesis is an important factor in the development of fatty liver in these animals.

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Biochemical and molecular diagnosis of Alpha-1-Antitrypsin deficiency in Iranian children affected by idiopathic liver dysfunction. *S. Zare Karizi¹, R. Mirfakhraie¹, M. Houshmand¹, T. Zaman², F. Mirzajani¹.* 1) medical genetics, National Institute of Genetic Engineering & Biotechnology, Tehran, Iran; 2) Children medical center, Tehran Medical University, Tehran, Iran.

75 families clinically characterized by idiopathic liver dysfunctions (eg. Cirrhosis and chronic hepatitis and/or respiratory problems such as early emphysema and bronchiectasy) have been referred to our center. Biochemical tests including SRID were performed to determine the enzyme concentration. PCR- RFLP method was used in order to detect the presence of Z and S mutations. Results from molecular diagnosis were compared with 1AT concentration obtained from Serum Radial Immuno Diffusion (SRID). The association of 1AT genotype with serum concentration was also determined.

Tissue-specific expression of alternatively spliced forms of human Xp21 glycerol kinase (GK) gene. *Y. Zhang, B.L. Huang, E.R.B. McCabe.* Depts of Pediatrics and Human Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA.

GK deficiency (GKD) is X-linked and results from mutation of the gene encoding GK. GK is involved in carbohydrate and fat metabolism, as well as glucocorticoid-glucocorticoid receptor nuclear uptake. There is poor correlation between genotype and GKD disease severity. To investigate the hypothesis that the protein encoded by the GK gene mapping to Xp21 has additional functions that influence phenotype, we examined four alternatively spliced forms of GK mRNA in different human tissues and cell lines: 1) with exon 8A and without exon 18 (GK+EX8A-EX18); 2) with exon 8A and exon 18 (GK+EX8A+EX18); 3) without exon 8A and with exon 18 (GK-EX8A+EX18); and 4) without exon 8A or exon 18 (GK-EX8A-EX18). Reverse transcriptase-polymerase chain reaction (RT-PCR) amplified human Xp21 GK exons 6 to 19 using primers within exon 5 and the 3'-untranslated region, and resulted in distinct products for each mRNA. After TA cloning, dot blot hybridization was performed with probes containing exon 8A or exon 18 sequence. Sequencing of one colony for each expressed transcript for each tissue or cell line confirmed the results. RT-PCR was done on human tissue polyA RNA and on total RNA extracted from cell lines. Human kidney and fetal liver only expressed GK-EX8A-EX18 (transcript 4). G402 cells (human renal leiomyoblastoma) expressed transcripts 1, 2 and 3 but not 4. HepG2 cells (human liver) expressed transcripts 3 and 4, but not 1 or 2. Cell lines SS6-2 (stem cell myotubes) and A549 (human lung) expressed transcripts 2, 3 and 4, but not 1. Human tissues, such as human adult brain, fetal brain, placenta, testes, and cell lines including human fibroblasts, G401 (human kidney rhabdoid tumor), HEK293 (human embryo kidney) and Cos7 (monkey kidney) expressed all transcripts, but with different intensities. We conclude that the four alternatively spliced GK mRNAs are differentially expressed. We speculate that these different expression patterns may indicate specific and possibly divergent functions for the individual isoforms. Genetic and environmental factors that alter these expression patterns in different individuals could influence the phenotypes of individuals with GKD.

Acylcarnitine Profile Alterations in Autistic Patients. *S. Carlo*¹, *N. Arciniegas*², *J. Acevedo*³, *M. Lopez*³, *C. Burgos*¹, *N. Suarez*¹, *R. Oliveras*¹, *V. Franceschini*¹, *A.S. Cornier*¹. 1) Genetics Division, Ponce Sch Medicine, Ponce, PR; 2) Department of Pediatrics, Ponce School of Medicine, Ponce, PR; 3) Department of Nursing, Univ. of Puerto Rico at Arecibo, Arecibo, PR.

Autism is a complex behavioral disorder defined completely on the basis of impairments in social interaction, impairments in communication, and repetitive and stereotypic behaviors. For most children, the onset of autism is gradual; however, but about 30% show regression of milestones specifically speech. Physical examination included a complete dysmorphology evaluation and an examination of the skin (including Woods lamp examination) to look for evidence of tuberous sclerosis complex and NF1. The dysmorphology examination should identify most, if not all, children with chromosomal disorders. Measurement of height, weight, and head circumference to asses appropriate growth was also evaluated. Metabolic samples were taken to evaluate lactate, pyruvate, urine organic acids, plasma amino acids and acylcarnitine profile and Fragile X DNA. We had previously reported elevated lactate and elevated lactate pyruvate ratio in patients with diagnosis of Pervasive Developmental Disorder and Autism. Further evaluation of these patients has shown unspecific elevations in the acylcarnitine profile. In our group of patients mild to moderate elevations of C-4, C-5 and C-10 were reported. We have also observed the same elevations in lactate, lactate-pyruvate ratio and acylcarnitines in patients with diagnosis of Fragile X syndrome. The elevations in the acylcarnitine profile, lactate and lactate-pyruvate ratio raises the possibility of a mitochondrial fatty acid oxidation disorder.

The Effects of Diffuse Traumatic Brain Injury on Mitochondrial Respiratory Complex Activities in the Immature Rat Brain. *B. Cobb*^{1,5}, *T. Chang*^{2,3}, *I.L. Cernak*⁴, *A. Faden*⁴, *L.J. Wong*¹. 1) Institute for Molecular and Human Genetics - Department of Oncology, Georgetown University, Washington, DC; 2) Neurology and Pediatrics, Childrens National Medical Center Washington, DC; 3) Neurology and Pediatrics, George Washington University School of Medicine, Washington, DC; 4) Neuroscience, Georgetown University Medical Center Washington, DC; 5) National Institutes of Health (National Human Genome Research Institute), Bethesda, MD.

Traumatic brain injury (TBI) is a leading cause of death and disability in both adult and pediatric populations. A new developmental model of diffuse TBI indicates different bioenergetic responses to injury in the early immature brain. Therefore, this study examines the respiratory enzyme activities and oxygen consumption rates in the mitochondria in the early immature rodent brain in response to diffuse TBI. Crude mitochondria extracts were isolated from 7 day old male Sprague Dawley (Harlan) rat brain tissue (cortex, subcortex, cerebellum, and hippocampus). The rat pups were injured and sacrificed at 4 and 24 hrs after injury and compared to nave pups. Citrate synthase activity decreased after injury in all four regions of the brain, particularly at 4 hrs post-injury. Comprehensive respiratory chain activity measurements demonstrates increased complex III activity at 4 hours post-injury when normalized to citrate synthase activity. This response is pronounced in the subcortex, cerebellum and hippocampus (3.83 2.31, 1.97 0.89, 2.16 0.04) compared to naves(0.92 0.05, 0.87 0.10, 0.51 0.10) or 24 hrs post-injury (0.97 0.37, 1.32 0.64, 0.3 0.65). These results suggest selective developmental vulnerability of the immature brain in response to injury. Interestingly, complex III is a site of ROS generation. Further studies are underway to investigate the effects of diffuse TBI on mitochondrial membrane potential, ROS production, and ATP production in the early immature brain. .

The mitochondrial DNA G13513A mutation is not a frequent cause of Leigh or MELAS syndrome. *F. Hol, M. Ruiten, M. Siers, C. van den Elzen, H. Scheffer.* Dept Human Genetics, Univ Medical Ctr, Nijmegen, Netherlands.

The G13513A mutation in the mitochondrial complex I subunit gene ND5 has previously been reported in patients with Leigh syndrome, MELAS syndrome and in a single patient with MELAS/LHON-overlap syndrome. Several authors have claimed that this mutation is a frequent cause of MELAS(-like) and Leigh(-like) syndromes (Pulkes et al. 1999; Chol et al. 2003) and that patients suspected to suffer from these syndromes should be routinely tested for this mutation. We selected 300 patients from our records displaying either Leigh-like or MELAS-like symptoms or were shown to have impaired complex I activity in muscle. All subjects were previously found to be negative for common mitochondrial pointmutations at positions 3243, 8344 and 8993. We designed a Pyrosequence assay that allows rapid testing for presence of the G13513A mutation and accurate determination of the level of heteroplasmy. All 300 subjects were tested for this mutation. Only two patients, both displaying a Leigh-like phenotype, were found to be positive. Patient 1 was a male diagnosed to have Leigh syndrome at the age of seven. He was mentally retarded with strabismus, optical atrophy and spastic paresis and he had typical lesions on CT-scan. At age seventeen he developed renal insufficiency for which he needed dialysis. At age 23 he received a hip prosthesis because of cox arthrosis and died shortly after surgery. The mutation was found to be present in muscle (63%) and blood (42%). The second patient is a female diagnosed to have Leigh syndrome at the age of nine. She is now 25 years old and the disease has progressed showing mental deterioration and neurological dysfunction. The mutation was found in muscle (65%) and blood (26%). The mutation was not detected in blood leucocytes of the asymptomatic mother and brother. In conclusion, our results do not support previous statements by others that the G13513A mutation is a frequent cause of Leigh and MELAS (-like) syndromes.

Deficiency in pantothenate kinase 2 in a mouse model for Hallervorden-Spatz Syndrome leads to retinal degeneration and azoospermia. *Y. Kuo*^{1,2}, *J. Duncan*¹, *S. Westaway*³, *H. Yang*¹, *G. Nune*¹, *E. Xu*¹, *S. Hayflick*³, *J. Gitschier*^{1,2}. 1) Univ California, San Francisco; 2) Howard Hughes Medical Institute; 3) Oregon Health and Science University.

Pantothenate-kinase associated neurodegeneration (PKAN, formerly known as Hallervorden-Spatz Syndrome) is a rare but devastating neurodegeneration syndrome, resulting from an inherited defect in coenzyme A biosynthesis. As pathology in the human condition is limited to the inaccessible structures in the CNS, including the retina and globus pallidus, we have generated a mouse knock-out of the orthologous murine gene (*Pank2*). We have discovered that homozygous null mice manifest changes in the eye. Specifically, *Pank2* mice show retinal degeneration with scotopic a-waves and b-waves significantly lower than those of wild-type littermates, decreased cell number and disruption of the outer segment, and reduced pupillary constriction response. The homozygous male mutants are infertile and suffer complete azoospermia, a condition that was not appreciated in the human. In contrast to the human, however, no changes were noted in the basal ganglia by MRI or by histological exam, nor were there signs of dystonia, even following the mice for one year. Dysphagia was also not apparent, although the *Pank2* mice are 20% decreased in weight compared to their wild-type littermates. Immunohistochemistry shows staining consistent with localization of *Pank2* to the mitochondria in both the retina and spermatocyte. In an attempt to elicit a movement disorder, mice were fed on diets deficient in pantothenate acid (B5-depleted) or B5 supplemented (control) diets starting at 5 weeks of age. While wild-type and *Pank2* animals fared poorly, with development of white fur patches, impaired movement and grooming, and decrease in body weights, the *Pank2* mice were more severely affected and died precipitously and simultaneously at 5 months. B5-depleted wild-type animals were sacrificed at 5 months for histological examination. No iron accumulation in the brain, retina or testes was detected, but the testes showed an absence of mature sperm and disorganization in the tubule, similar to the *Pank2* knock-out animals.

Novel mtDNA G3242A and G3244A mutations adjacent to a common A3243G mutation. *M. Mimaki*¹, *I. Nishino*², *I. Nonaka*², *Y. Goto*¹. 1) Department of Mental Retardation and Birth Defect Research, National Institute of Neuroscience, NCNP, Tokyo, Japan; 2) Department of Neuromuscular Research, National Institute of Neuroscience, NCNP, Tokyo, Japan.

Mutations in mitochondrial tRNA Leu (UUR) gene demonstrate marked phenotypic variability, including conditions as diverse as mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS). We identified two patients with mitochondrial disorders having a novel mutation in this mitochondrial tRNA gene, a transition at nucleotide position 3242 and 3244, respectively. First patient was a six-year-old girl with typical symptoms of MELAS. Muscle biopsy also showed typical findings of MELAS such as ragged-red fibers (RRF) and strongly SDH-reactive blood vessels (SSV). Total mitochondrial DNA sequencing revealed a heteroplasmic G3242A mutation. Fibroblasts derived from the patient showed complex I deficiency, and the threshold effect on respiratory chain enzyme activities in transmitochondrial cell lines confirmed the pathogenesis of this mutation. Second patient was an eleven-month-old female with lactic acidosis, cardiomyopathy, and renal tubular dysfunction without typical symptoms of MELAS. Muscle biopsy suggested mitochondrial abnormalities but neither RRF nor typical SSV was detected. Fibroblasts derived from the patient showed normal respiratory chain enzyme activities. Sequencing of DNA from muscle and fibroblasts revealed the essentially homoplasmic G3242A mutation, however, it was not detected in blood of her healthy mother, which suggests pathogenicity of the mutation. Although both of these two mutations were adjacent to an A3243G mutation, the most common mutation associated with MELAS, phenotypes of two patients and biochemical effects were quite different. Investigation regarding different effects by the G3244A and G3242A mutations on the phenotypes and similarities shared by the A3243G and G3244A mutations may provide a better understanding of tRNA dysfunction as well as an insight into the complicated issues surrounding the correlation between genotype and phenotype in mitochondrial disorders.

Role of mannose-6-phosphate receptor (M6PR300) in enzyme uptake and glycogen clearance in Pompe disease.

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Pompe disease, a lysosomal glycogen storage disorder resulting from acid -glucosidase (GAA) deficiency, is amenable to enzyme replacement therapy (ERT). MPR300 is necessary for lysosomal enzyme uptake *in vitro*, however it is presently unclear if the same system is involved *in vivo*. We have generated viable tissue-specific MPR300 knockout (KO) mice to investigate this question. To further elucidate the role of MPR300 in recombinant human GAA (rhGAA) uptake and glycogen clearance in Pompe disease, muscle-specific MPR300 KO mice were crossed with GAA KO (Pompe) mice to create double transgenic mice. These mice were administered four weekly doses of 20 or 100mg/kg of rhGAA and sacrificed 3 days after the last dose. Age-matched Pompe mice and mice administered with vehicle were used as controls. Enzyme activity results demonstrated decreased enzyme uptake in the skeletal tissues (quadriceps, gastrocnemius and triceps) and heart of the double KO mice as compared to the Pompe mice (80, 70 and 76 and 50% reduced uptake respectively). Glycogen content data indicated that glycogen clearance in the heart was 77% in Pompe mice as compared to 7% in double KO mice at 20mg/kg dose, although similar glycogen clearance was seen in both mouse models at 100mg/kg dose. In the skeletal muscle, the glycogen depletion was significantly decreased in the double KO mice compared to the Pompe controls (28, 27 and 33% reduction in the double KO mice for the quadriceps, triceps and gastrocnemius respectively, compared to 71, 72 and 74% in Pompe mice). Our data suggests that skeletal muscle and the heart seem to be largely dependent on M6PR for rhGAA enzyme uptake and glycogen clearance *in vivo*, although the heart appears to employ alternative mechanisms for enzyme uptake and glycogen depletion at higher doses of rhGAA besides M6PR. Muscle-specific MPR300 KO mice are an effective animal model to better define the lysosomal enzyme uptake and targeting *in vivo* and this may lead to improvements in ERT currently available for Pompe and other lysosomal storage diseases.

The CPSI T1405N polymorphism is an independent predictor of postoperative pulmonary hypertension in children following congenital heart surgery. *J.A. Canter, M.L. Summar, H.B. Smith, K. Christian, D. Drinkwater, J.L. Haines, F.E. Barr.* Vanderbilt University Medical Center, Nashville, TN.

Pulmonary hypertension (PHTN) is especially dangerous to children following surgical repair of congenital heart anomalies. Added pulmonary vascular resistance, in the setting of decreased right ventricular contractility, creates a perilous low-cardiac output state. Extreme interventions, including inhaled nitric oxide (NO), are required to treat this common complication. In this study, we followed a consecutive cohort of children (N=131) with congenital heart defects requiring open-heart surgery to determine key factors in the development of postoperative PHTN. All children in this cohort had mean pulmonary artery pressure (MPAP) measured by indwelling pulmonary artery catheters placed during surgery. PHTN was defined as a MPAP >20mmHg for at least one hour during the 48 hours following surgery despite pharmacologic intervention. In this cohort, 59% (78/131) children had postoperative PHTN. Age, cardiopulmonary bypass time, and presence of Down syndrome were expected to contribute to this complex phenotype. We also determined the frequency of a well-characterized polymorphism in the CPSI gene, T1405N, that has previously been implicated in the availability of substrate for endogenous NO production. The precursor of endogenous NO is arginine supplied by the urea cycle. CPSI encodes for the enzyme, carbamoyl-phosphate synthetase, which catalyzes the rate-limiting step in the urea cycle. We found a significant difference in the CPSI T1405N polymorphism frequency in patients developing postoperative PHTN compared to those without PHTN (P=0.03). The logistic regression model constructed from these variables (log likelihood = -72.8) revealed that age (OR=0.92, 95% CI 0.87-0.98), presence of Down syndrome (OR=5.24, 95% CI 1.11-24.77) and CPSI T1405N genotype (CC genotype vs. AC genotype, OR= 4.08, 95% CI 1.04-16.04 and CC genotype vs. AA genotype, OR= 5.97, 95% CI 1.54-23.15) were independent predictors of postoperative PHTN. Accurate prediction of susceptibility to postoperative PHTN will likely need to take into account the genetic variation in urea cycle capacity.

GNE enzyme activity in Hereditary Inclusion Body Myopathy. *S. Sparks*¹, *C. Ciccone*¹, *M. Lalor*¹, *E. Orvisky*², *D. Krasnewich*¹, *M.-S. Sun*¹, *M. Dalakas*³, *W. Gahl*¹, *M. Huizing*¹. 1) SHBG, MGB, NHGRI, NIH, Bethesda, MD; 2) NBS, NIMH, NIH, Bethesda, MD; 3) NINDS, NIH, Bethesda, MD.

UDP-GlcNAc 2-epimerase/ManNAc kinase, encoded by *GNE* on 9p12-13, is the bifunctional and rate-limiting enzyme in the biosynthesis of sialic acid. The epimerase converts UDP-GlcNAc to ManNAc and the kinase converts ManNAc to ManNAc-6-phosphate. Mutations in either or both the epimerase and kinase domains of *GNE* cause Hereditary Inclusion Body Myopathy (HIBM), an autosomal recessive disorder characterized by adult-onset, progressive distal and proximal muscle weakness sparing the quadriceps muscles. We employed cultured fibroblasts and a cell-free transcription/translation system to investigate the epimerase and kinase activities of 3 HIBM patients and their individual *GNE* mutations. The cell-free system allows the measurement of GNE activity without influences from other enzymes present in the fibroblasts.

In cultured fibroblasts from HIBM patients, residual epimerase activity ranged from 38% to 83% of normal. However, when the same patient mutations were cloned into *GNE* and the resulting protein translated in the cell-free system, the GNE-epimerase activity was negligible compared to wild type *GNE*. We conclude that fibroblasts contain enzymes, besides GNE, that convert UDP-GlcNAc to ManNAc. Kinase activity in cultured fibroblasts from an HIBM patient with a homozygous 2186T>C mutation was 26% of normal. When this same mutation was cloned into *GNE* and expressed in the cell-free system, the GNE-kinase activity was 28% of normal. This indicates that there is residual *in vivo* kinase activity in HIBM patients, and this activity may be necessary for survival. Further evidence of this is the embryonic lethality of the *GNE* knock-out mouse.

Comparison of GNE epimerase and kinase activity in both systems will help to explain the phenotype of HIBM patients with specific mutations in the epimerase or kinase domains.

Mutation Analysis of 25 Colombian Gaucher Disease Patients using dHPLC: Report of three novel mutations.

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Gaucher Disease is the most common of the lysosomal storage disorders, affecting all ethnic groups. The pathology of this recessively inherited disease arises from the accumulation of glucocerebroside in tissues due to deficient activity of the enzyme glucocerebrosidase (E.C. 3.2.1.45). The glucocerebrosidase (GBA) gene spans a 7.2 kb fragment located on locus 1q21, consisting of 11 exons and 10 Introns. Located 16kb downstream is a highly homologous pseudogene sequence (Horowitz, 1989). Fourteen fragments comprising 11 exons of the GBA gene were analyzed in DNA samples from 25 Colombian patients using denaturing High Pressure Liquid Chromatography (dHPLC). Sequencing of abnormal findings led to the discovery of three novel mutations (595-596delCT, 898delG and N380H in exons 6, 7 and 9 of the GBA gene) with high prevalence among Colombian patients. We have also found the presence of a double mutation (L444P-E326K) in two different families classified as Gaucher Type 1. This mutation was previously reported in one patient with Gaucher type 2.

Identification of Seven Novel Mutations in the Alpha Glucosidase Gene in Argentinian Patients with Glycogen Storage Disease Type II. *N.A. Chamoles¹, R.E. Palmer², R.J. Pomponio².* 1) Neurochemistry Laboratory, Buenos Aires, Argentina; 2) Pharmacogenetics, Clinical Laboratory Science, Genzyme Corporation, Framingham, MA 01701.

Glycogen Storage Disease II (GSDII) is an autosomal recessive disorder caused by a deficiency in acid alpha glucosidase (GAA), the enzyme required to hydrolyze lysosomal glycogen to glucose. In the absence of sufficient quantities of functional enzyme, glycogen accumulates within lysosomes resulting in cardiac and skeletal muscle dysfunction. Disease severity varies among patients and is differentially classified as either infantile, juvenile, or adult, based on age of onset and clinical severity. From 12 Argentinian patients who were diagnosed with various forms of GSDII based on biochemical analysis of GAA function, we have identified 7 novel mutations (236_246del, 377G>A, 1397T>G, 1755-1G>A, 1802C>G, 1978C>T, and 2608C>T). Three different families displayed the g.377G>A allelic variant encoding a truncated form of GAA (p.Trp126Ter), suggesting a high frequency among a subset of Argentinians. Additionally, we identified a single patient presenting with juvenile onset disease who was heterozygous for g.1978C>T encoding for p.Arg660Cys. A previous association with an amino acid substitution at this position (Arg660His) and juvenile disease in two Japanese patients suggests a possible correlation with changes at this amino acid and less severe disease. The identification of novel mutations, as well as supporting evidence for previously identified genotype/phenotype associations, demonstrates the importance of continued mutational analysis in populations from diverse geographic locations.

Mutation and biochemical analysis of patients belonging to the *cbIB* complementation class of vitamin B12-dependent methylmalonic aciduria. *J.P. Lerner-Ellis*¹, *C.M. Dobson*⁵, *T. Wai*¹, *D. Watkins*¹, *J.C. Tirone*¹, *C. Dore*⁴, *P. Lepage*⁴, *R.A. Gravel*⁵, *D.S. Rosenblatt*^{1,2,3,4}. 1) Human Genetics, McGill University, Montreal, Quebec, Canada; 2) Biology, McGill University, Montreal, Quebec, Canada; 3) Medicine, McGill University, Montreal, Quebec, Canada; 4) McGill University and Genome Quebec Innovation Centre, Montreal, QC, Canada; 5) Department of Biochemistry and Molecular Biology, University of Calgary, AB, Canada.

The *cbIB* disorder is a rare inborn error of vitamin B₁₂ (cobalamin, Cbl) metabolism that occurs due to mutations in the *MMAB* gene, which encodes the enzyme ATP:cobalamin adenosyltransferase. This disorder results in decreased synthesis of the cobalamin derivative adenosylcobalamin (AdoCbl). AdoCbl is required for activity of the mitochondrial enzyme methylmalonyl CoA mutase (MCM), which catalyzes the isomerization of methylmalonyl CoA to succinyl CoA. Deficient MCM activity results in methylmalonic acidemia and methylmalonic aciduria and a susceptibility to life-threatening acidotic crises. Seven mutations in the *MMAB* gene have previously been identified in *cbIB* patients. In this study, we have investigated a panel of eighteen *cbIB* patients. Cultured fibroblasts from all patients had decreased synthesis of AdoCbl from exogenous labeled cobalamin and decreased incorporation of [¹⁴C] propionate into cellular macromolecules, an indirect measure of MCM activity. Diagnosis of the *cbIB* disorder was confirmed in all cases by complementation analysis. Genomic DNA from patients was analyzed for deleterious mutations in the *MMAB* gene. Six novel mutations were identified, and of the seven previously identified mutations, four were found in at least one additional patient. None of these mutations were identified in one hundred control alleles. In total, thirteen mutations have now been identified in eighteen *cbIB* patients.

Identification of 6 Novel Mutations in the Acid Alpha Glucosidase Gene Associated with Infantile-Onset Pompe Disease. *R.E. Palmer¹, J. Abysalh², C. Braithwaite², A. McPhee², L. Smith², L. Atwater², C. Sung², S. Richards², R.J. Romponio¹.* 1) Clinical Laboratory Science, Pharmacogenetics, Genzyme Corporation, Framingham, MA. 01701; 2) Department of Immunology, Genzyme Corporation, Framingham, MA 01701.

Pompe disease (Glycogen storage disease II) is an autosomal recessive lysosomal storage disease resulting from mutations within the acid alpha glucosidase gene (GAA). Patients with Pompe disease accumulate lysosomal glycogen and display a spectrum of disease severities. The most severely affected infantile-onset patients present with generalized muscular weakness and cardiac hypertrophy that lead to death before 1 year of age. We have evaluated a group of 10 infantile-onset patients for mutation status and GAA protein expression and have identified twenty distinct GAA mutations including six that are novel. Patients carrying missense mutations on one or both alleles express varying levels of processed and unprocessed GAA suggestive of some functional heterogeneity. A single patient with two truncating mutations has no detectable protein supporting previous findings that truncated forms of GAA are not expressed. One novel mutation results in loss of the initiator methionine of the protein (g.3G>A). Western blot analysis of protein derived from patient fibroblasts reveals low levels of aberrantly sized products that may represent use of alternative translational initiation sites. This analysis demonstrates that, although over 100 different disease-associated GAA variants have already been identified, continued genotyping efforts yield novel findings that may enhance our understanding of genotype/phenotype associations.

Mutation spectrum of the human ATP7B gene of Korean patients with Wilson Disease. *S-W. Park¹, G-H. Kim¹, H-W. Yoo^{1, 2}*. 1) Genome Research Center for Birth Defects & Genetic Disorders, Asan Medical Center, Seoul, Korea; 2) Department of Pediatrics, Asan Medical Center, Univeristy of Ulsan College of Medicine, Seoul, Korea.

Wilson disease (WD) is an autosomal recessive disorder of copper transport, probably one of the most common inherited metabolic disorder in Korea. The product of WD gene is a copper transporting P-type ATPase(ATP7B). In this study, efforts have been made to identify novel mutations and investigate an allele frequency of each mutation in Korean patients with WD. This study includes 115 unrelated Korean patients diagnosed as WD by clinical and biochemical findings; a low serum ceruloplasmin (<15mg/dL) and increased urinary copper excretion (>100ug/day). Initially, direct genomic DNA sequencing of PCR products of exon 8, 11, and 16 was performed to screen common mutations (R778L, A874V, N1270S) in Korean WD patients, subsequently the exons 10,13,14,15 and other exons were analyzed. If no mutation was found, promoter region was evaluated. We identified 23 different mutations in 115 unrelated Korean patients with WD: R778L, A874V, N1270S, c.2513delA, G1035V, T1029I, L1083F, c.2630-2656del, c.2304-2305 insC, IVS3 +1 G>T, V1106I, M729V, D1267A, C656X, A1168S, G943S, R919G, I1148T, G891D, P992L, G1186S, and T1031A. Among these mutations, seven mutations were novel; C656X, M729V, G891D, V1024A, T1029I, T1031A, V1106I. We characterized molecular defects of the ATP7B gene in only 72% of Korean Wilson patients. The R778L is the most common mutation with 38.7% of allele frequency that is more prevalent than previously reported in Korean patients. The other common mutations are A874V, N1270S and 2513delA. Their allele frequencies are 7.4, 5.6, and 4.0% respectively. Most patients carrying aforementioned mutations are compound heterozygotes, which hinders making a genotype-phenotype correlation. In conclusion, the spectrum of mutations in the ATP7B gene of Korean patients with Wilson disease is very heterogeneous, although the R778L mutation is the most common.

Persistence of 3-hydroxyisovaleryl carnitine in blood spots of infants born to mothers with 3-methylcrotonylglycinuria. *A.A. Basinger, D.M. Frazier, J. Muenzer.* Pediatrics, University of North Carolina, Chapel Hill, NC.

Expanded newborn screening with tandem mass spectroscopy allows detection of additional inborn errors of metabolism in infants, and in some cases, mothers of screened infants. The most common organic aciduria identified by newborn screen (NBS) in North Carolina (NC) is 3-methylcrotonyl-glycinuria, caused by deficiency of 3-methylcrotonyl-CoA carboxylase (3-MCC), an enzyme in the degradation pathway of leucine. Since 1997, 801,744 children have been screened by the NC NBS program and 17 identified with 3-MCC deficiency. A blood spot (BS) 3-hydroxyisovaleryl (C5-OH) carnitine of 2.60 M on initial NBS or 1.36 M on initial and repeat NBS suggests 3-MCC deficiency. In NC, confirmatory testing includes plasma acylcarnitine profile, carnitine, urine organic acids, and biotinidase. Six previously undiagnosed women were identified with 3-MCC deficiency after their infants had positive NBS and negative confirmatory testing. Infants of affected mothers had initial BS C5-OH carnitines of 6.312.61 M and repeat values of 5.381.81 M, similar to infants with confirmed 3-MCC deficiency (5.023.71M). BS C5-OH carnitine increased in 4/9 children of affected mothers between the initial and repeat NBS. Elevated BS C5-OH carnitines persisted in three infants up to 2 months. Mothers' levels of BS C5-OH carnitine were 8.12-33.0 M. Four mothers reported good general health. One had chronic joint pain and fatigue that responded to supplemental carnitine and a protein-restricted diet. One reported occasional episodes of emesis with mild illnesses and after high-protein meals. Proposed mechanisms for persistence of BS C5-OH carnitine in infants of affected mothers include delayed maturation of 3-MCC or intracellular retention of C5-OH carnitine. Of 23 infants identified with elevated C5-OH carnitine on initial and repeat NBS, 6 (26%) were infants of mothers with 3-MCC deficiency. The addition of a maternal BS acylcarnitine profile to confirmatory testing would allow prompt differentiation between infant and maternal 3-MCC deficiency.

Cerebral complications and treatment of Cobalamin C deficiency. *V. Cunningham¹, P. Laoprasert¹, M. Matthews¹, T. Bottiglieri², K. Hyland², S. Stabler¹, S. Zeisel³, C. Freehauf¹, J. Van Hove¹.* 1) Depts of Pediatrics, Pathology and Medicine, Univ of Colorado Health Sciences Ctr, Denver, CO; 2) Institute of Metabolic Disease, Baylor Univ Med Ctr, Dallas, TX; 3) Dept of Nutrition, School of Medicine, Univ of North Carolina, Chapel Hill, NC.

Cobalamin C disease is a disorder of vitamin B12 metabolism with methylmalonic (MMA) aciduria and homocystinuria. Current treatment with parenteral hydroxycobalamin (OH-Cbl), oral betaine and folate improves biochemistry and neurologic outcome but in infants mental retardation is still frequent. Here we explored reasons for this poor outcome. A 2 week old male presented with hard-to-control seizures, methylmalonic aciduria, and hyperhomocysteinemia. Fibroblast studies confirmed cobalamin C deficiency. A gyral cortical malformation was identified on EEG, MRI, and ictal PET studies and he underwent partial frontal cortex resection for seizure control. Pathology showed cortical dysplasia with severe neuronal migrational defects. We did not identify previous literature reports of cortical migrational defects in this disorder. Without such extensive studies these malformations may be under-recognized. Initial therapy of OH-Cbl 1 mg/d (285 g/kg/d) IM, and betaine 200 mg/kg/d reduced serum MMA from 21,455 to 1595 nmol/l (normal 73-271) and homocysteine from 140 to 21 mol/l (normal 4-12). Maintaining betaine but increasing OH-Cbl up to 5 mg SC daily did not further reduce serum MMA and homocysteine. CSF analysis on initial therapy showed elevated MMA 23,299 nM (normal 73-271), and homocysteine 1238 nmol/L (normal 32-79), but reduced methylfolate 31 nmol/L (normal 40-187) and choline 1.27 mol/l (normal 1.4-2.2), indicating inadequate biochemical control behind the blood-brain-barrier. Options for improvement of cerebral biochemistry may include folinic acid and choline substitution, and higher OH-Cbl doses. On added choline 125 mg/kg/d and folinic acid 5 mg/d, and on 1 mg/d (178 g/kg/d) OH-Cbl and 135 mg/kg/d betaine, CSF showed increased MMA 73,315 nM, decreased homocysteine 697 nmoles/L, and improved methylfolate 182 nmol/L. Such strategies should be explored to improve cerebral biochemical control and neurologic outcome.

Branched chain amino acid metabolites in plasma and urine acylcarnitine analyses. *S.E. McCandless¹, P.E. Minkler², M.K. Stoll², C.L. Hoppe^{2,3}*. 1) Center for Human Genetics, Case Western Reserve University and University Hospitals of Cleveland, Cleveland, OH; 2) Medical Research Service, Louis Stokes Department of Veterans Affairs Medical Center, Cleveland, OH; 3) Departments of Pharmacology and Medicine, Case Western Reserve University School of Medicine, Cleveland, OH.

Two recently identified deficiencies (isobutyryl-CoA dehydrogenase and 2-methylbutyryl-CoA dehydrogenase) involve the metabolism of the branched chain amino acids valine and isoleucine. The markers identified in MS/MS newborn screening programs for these disorders are isomers of C4 and C5 acylcarnitines. Our laboratory uses HPLC/MS to resolve and identify these and other isomeric acylcarnitines that can't be resolved by MS/MS. We use standardized solutions of isobutyrylcarnitine (IB) and 2-methylbutyrylcarnitine (2-MB) to generate multiple point standard curves, allowing quantitation of IB and 2-MB in patient samples. Initially, the normal range was defined by samples from a small number of normal adult volunteers. We now present data from accumulated samples showing the range of values seen in patients referred for evaluation (presumably most with some clinical indication). IB was found in roughly half of the plasma samples (range 0 to 2.15 mol/L, 97th %ile 0.38 mol/L, N=1806), and in 90% of urine specimens (range 0 to 156.3 mol/g creatinine, 97th %ile 39.8, N=1117). None of the samples were from patients known to have isobutyryl-CoA dehydrogenase deficiency, although the definitive diagnosis was not determined in all cases. More samples (88%) had 2-MB in plasma (range 0 to 2.89 mol/L, 97th %ile 0.36, N=1706), while 92% of urine samples had measurable quantities (range 0 to 260.5 mol/g creatinine, 97th %ile 29.9, N=1062). It is important to note that the plasma 2-MB concentration in a patient with known deficiency was elevated, but the value was similar to values seen in individuals on L-carnitine therapy for a variety of other disorders. This study shows that the range of values seen for these metabolites in many of the samples referred to the laboratory is higher than expected based on a small number of normal controls.

Genetic and Genomic Systems to Study Methylmalonic Acidemia (MMA). *C. Venditti*¹, *R. Chandler*¹, *M. Tsai*², *N. Wehrli*², *R. Deering*², *K. Kaestner*², *G. Giaever*³, *H. Kazazian*². 1) GDRB, NHGRI/NIH, Bethesda, MD; 2) University of Pennsylvania, Philadelphia, PA; 3) Stanford Genome Technology Center, Palo Alto, CA.

Nematode, yeast and murine models have been developed to study methylmalonic acidemia. We identified homologues of PCCB, MMAA, MMAB, MMCM, and MMCR in *C. elegans* and cloned them as full-length cDNAs for feeding interference. RNAi directed against MMAA, MMAB, and MMCM caused the animals to produce >30,000 nM methylmalonic acid (MMA) in the incubation media after interference and substrate loading, which corresponded to a 4-5 fold increase over WT. This system can be used to examine chemical and genetic modifiers of propionyl-CoA metabolism and to identify cobalamin metabolic candidate genes using the genomic resources of *C. elegans*. To identify cellular targets of MMA toxicity, we performed chemogenomic profiling using yeast exposed to either three percent or 100 uM MMA. Genes encoding MMA sensitivity loci were identified using whole genome parallel analysis. A diverse set of >50 mutants, corresponding to known or predicted yeast genes, identified potential targets of MMA toxicity. These include proteins involved in vesicle formation, gluconeogenesis, neutral amino acid transport and the glycine cleavage system. Two mutants displayed extreme sensitivity to MMA. One encodes a vacuolar H-ATPase regulatory subunit b that is mutated in a human syndrome of distal renal tubular acidosis and deafness. The other has homology to phosphoglycerate mutase, a key enzyme of gluconeogenesis and glycolysis. These mutants may help understand the perturbation of intermediary metabolism seen in MMA and have allowed a simple yeast growth assay, demonstrating concentration dependent MMA toxicity, to be created. This system will enable yeast genetics to be used to study MMA toxicity. Finally, we have created a mouse model of methylmalonyl-CoA mutase deficiency. The mice display many features seen in the human condition, including massive elevation of MMA in body fluids, and perish shortly after birth. These diverse model organism systems will allow the human condition to be studied using modern genomic and genetic methods, and should facilitate the development and testing of new therapies for MMA.

Molecular analysis of Peroxisome Biogenesis Disorders. *N. Shimozawa*^{1,2}, *T. Nagase*², *Y. Suzuki*³, *N. Kondo*², *R.J.A. Wanders*⁴. 1) Life Science Research Center, Gifu University, Gifu, Japan; 2) Department of Pediatrics, Gifu Univ Sch of Med, Gifu, Japan; 3) Medical Educat Develop Center, Gifu Univ Sch of Med, Gifu, Japan; 4) Dept of Clin Chem and PEDIATR, Univ of Amsterdam, Netherlands.

Peroxisome biogenesis disorders (PBD) are genetically heterogeneous diseases with an autosomal recessive inheritance. We have analyzed the PBD at the molecular level, as the only diagnostic center for peroxisomal disorders in Japan. (1) We identified a total of 32 Japanese PBD patients, those were subdivided into 6 complementation groups (CG). (2) We demonstrated two common mutations: a G89R mutation of PEX26 in all patients from CG-A and a 2 base pair deletion of PEX10 in all patients from CG-B, homozygously. To determine whether the high frequency of these mutations are due to a founder effect, we analyzed SNPs in these genes among the patients and Japanese controls, which suggested that both mutations arose once on an ancestral chromosome in the Japanese population, respectively. (3) So far, 12 different CG of PBD were identified in the world. To date, we have identified a new CG-K of PBD with PEX14 as the defective gene. Human PEX14 rescues the import of a PTS1-dependent as well as a PTS2-dependent protein into the peroxisomes in fibroblasts from a patient with Zellweger syndrome belonging to the CG-K. This patient was homozygous for a nonsense mutation in a putative coiled-coil region of PEX14, Q185X. We showed that the patients fibroblasts lacked PEX14 as determined by immunocytochemical analysis. These findings indicate that there are 13 genotypes in PBD and that the role of PEX14 is also essential in humans.

Glucocorticoid receptor gene polymorphisms: frequencies and impact on ALL outcome. A. Gahier-Rudolf, A. Doreau, D. Labuda, D. Sinnott, A. Moghrabi, M. Krajcinovic. Hemato-oncology dept, Research center, Ste-Justine hospital, Montréal, Canada.

Corticosteroids (CS) modulate a large number of physiological functions that, along with their anti-proliferative effect, explain the wide therapeutic spectrum of synthetically derived counterparts. The acute lymphoblastic leukaemia (ALL) is the most frequent pediatric cancer, whose treatment beside other anti-tumor agents, comprises CS drugs as an important component. However, the response to CS is not always optimal: at relapse, loss of *in vitro* sensitivity to CS is common, whereas various side effects have been also reported. CS exert their action through binding to the glucocorticoid receptor (GR), which then acts as a transcriptional regulator of responsive genes rendering GR polymorphisms potential modulators of CS response. We have previously shown that Bcl I polymorphism in GR locus correlated with overall survival probabilities in childhood acute lymphoblastic leukemia (ALL) while Arg23Lys and Asn363Ser polymorphisms did not seem to play a role. In order to further explore the variability of this gene and its impact on ALL outcome, the regulatory gene region (2 kb upstream of the transcription initiation site) was searched for the sequence variability by dHPLC, followed by direct sequencing. Four polymorphisms comprising C to T, A to C, G to A and A to G base substitutions were identified. Analysis of DNA samples of 174 individuals from a number of populations (Africans, Asians, Amerindians, Middle-Easterners, and European) revealed low frequency (below 1%) for the first two base substitutions. Of interest were a G to A transversion confined only to Africans (2.9%), and an A to G polymorphism with frequencies of 5%, 2.9% and 1.4 % in Europeans, MiddleEasterners and Africans, respectively. The latter polymorphism was subsequently analyzed in 237 children of European descent that were treated for ALL at Ste-Justine Hospital, Montreal. The GG genotype was associated with reduced relapse free survival probabilities ($p=0.03$) indicating its potential for the prediction of recurrent disease (supported by Cancer Research Society and Genome Quebec).

Pharmacogenetic analysis of multiple sclerosis: identification of potential response markers for Glatiramer

Acetate treatment. *I. Grossman*^{1,2}, *N. Avidan*², *C. Singer*^{2,3}, *D. Goldstaub*³, *L. Hayardeny*³, *E. Ben-Asher*², *M. Chemla*², *T. Paperna*¹, *J.S. Beckmann*⁴, *D. Lancet*², *A. Miller*¹. 1) Division of Neuroimmunology and MS Center, Rappaport Faculty of Medicine, Technion, Haifa; 2) Department of Molecular Genetics, Weizmann Institute of Science, Rehovot; 3) TEVA Pharmaceutical Industries Ltd. Kiryat Nordau, Netanya; 4) Service of Medical Genetics, Centre Hospitalier Universitaire Vaudois, Lausanne.

Evidence is accumulating to indicate that response patterns to drugs are at least partly under genetic control. For a disease such as Multiple Sclerosis the evaluation of patients' response to drug treatment may take up to 2 years. During this time a significant deterioration in health may amass. For this reason, a diagnostic tool that will allow the allocation of drugs and their doses, based on objective genetic screening, may have a considerable impact on patient-care and health management. The field of pharmacogenetics is aimed at seeking such solutions by testing the potential associations between genetic markers, such as single nucleotide polymorphisms (SNPs) or their haplotypes, and drug response. In an exploratory study with this future goal in mind, we have genotyped 63 SNPs within 27 candidate genes likely to be involved in the presumed mode-of-action of the Multiple Sclerosis drug Glatiramer Acetate and its clinical response features. DNA samples were obtained from two different clinical trials, each of which was analyzed separately since they involved different populations and primary clinical endpoints. A number of candidate genes were found to be significantly associated with different response definitions. An overlap of associations between the two independent cohorts tested corroborates the robustness of the results. The fact that significant association is found despite the rather limited number of patients and SNPs studied, may indicate that the Glatiramer Acetate drug response is under the genetic control of a limited number of genes, each potentially having a rather pronounced effect. Taken together, our results indicate that the goal of personalized treatment for multiple sclerosis and possibly other autoimmune diseases may be within reach.

The influence of three polymorphisms of thymidylate synthase gene on the TS mRNA levels. *M. Primeau*¹, *M. Krajinovic*^{1,2}. 1) Hemato-oncology dept, Centre de recherche de l'Hôpital Sainte-Justine, Montréal; 2) Pediatrics dept, Université de Montréal; Montréal, Québec, Canada.

Interindividual variability in response to TS inhibitors could be caused by variable levels of thymidylate synthase (TS). A polymorphism in the 5UTR consisting of either two or three 28bp repeats modulate TS mRNA expression. Individuals that are homozygous for the triple repeat (3R) were found to have elevated RNA and protein levels compared with double repeat (2R) homozygotes. The presence of additional USF binding site seems to explain the higher transcriptional activity of 3R allele. A GC polymorphism within the second 28bp repeat element has been recently described. This substitution changes a critical residue in the USF consensus element rendering 3RC and 2R alleles with comparable transcriptional activity, as shown *in vitro*. In addition, a 6bp deletion in 3UTR seems to affect TS mRNA stability, decreasing mRNA levels. With the aim to understand the underlying mechanism of genotype-phenotype correlation in leukemia clinical settings, we analyze the impact of three reported TS polymorphisms on TS mRNA levels. Both normal and leukemia blood cells originating from 48 healthy donors and 32 patients with ALL were analyzed. The genotyping was performed by PCR followed by gel electrophoresis (TS repeat), enzyme digestion (GC polymorphism) or ASO (6bp deletion). TS mRNA levels were assessed by real-time PCR standardized to the expression of 2-microglobulin. The comparison between groups was done by ANOVA. No correlation was found between 3R allele or 6bp deletion and TS mRNA levels in either normal or leukemia cells. To explore the effect of tandem-repeat and GC polymorphisms on TS expression, the genotypes were combined according to the number of USF sites. The individuals whose genotypes contain 3 USF sites tended to have slightly higher mRNA levels ($p=0.08$) compared to those with 2 sites. The difference was seen in ALL samples only. High intra-group variability suggests the presence of additional factors relevant for TS expression.

VKORC1 Haplotypes Predictive for Warfarin Dose. *M. Rieder¹, A. Reiner¹, D. Nickerson¹, D. Blough¹, J. Smith¹, D. Veenstra¹, B. Gage², H. McLeod², C. Eby², A. Rettie¹.* 1) University of Washington, Seattle, WA; 2) Washington University, St. Louis, MO.

Warfarin is the definitive treatment for the long-term prevention of thromboembolic events, but poses considerable dose management problems because of its narrow therapeutic range, ethnic differences in dose requirements and inter-individual variability in dosing. Recently, the pharmacologic target for warfarin, vitamin K epoxide reductase (VKORC1) was identified. We hypothesized that SNPs in VKORC1 may affect the warfarin dose for patients on chronic warfarin therapy. An extended genomic region surrounding the VKORC1 gene (11.2 kb) was resequenced in 186 European American warfarin patients, who were treated to establish a therapeutic warfarin dose, and multi-ethnic controls (119 European Americans, 96 African Americans and 120 Asian Americans). We identified ten noncoding, common SNPs (>5% MAF) and inferred haplotypes in both the European clinical and control cohorts. Five common haplotypes were identified in the European clinical samples and multiple linear regression was used to evaluate their association with daily warfarin dose. Four haplotypes (H1, H2, H7, and H9) were significantly associated ($p < 0.05$) with warfarin dose. Furthermore, these haplotypes were grouped into clades A (low warfarin dose) and B (high warfarin dose), containing haplotypes H1 and H2 (clade A) and H7 and H9 (clade B), respectively. Clade diplotypes (AA, AB, BB) assigned to each patient were also significantly associated with warfarin dose (AA=2.70.8 mg/d, AB=4.91.9, BB=6.22.9; MeanSD). Overall, the proportion of warfarin dose variance explained by VKORC1 clades A and B was 25%. These VKORC1 results were replicated in an independent cohort ($n = 327$) of European American patients. We also identified a haplotype distribution in Asian and African American populations that is consistent with their lower and higher warfarin dose phenotypes, respectively. Prospective genotyping for VKORC1 SNPs and haplotypes may lead to more accurate initial dosing and quicker time to stable anticoagulation, thereby improving the safety, effectiveness, and costs of warfarin therapy.

CYP2B6 Genotype and Nicotine Metabolism. *H.Z. Ring¹, A.M. Valdes¹, D. Katsuyoshi¹, P. Jacob², R. Walton³, G.E. Swan¹, N.L. Benowitz².* 1) SRI International; 2) University of California, San Francisco; 3) University of Oxford.

CYP2B6 metabolizes nicotine and many therapeutic drugs including bupropion. An amino acid changing polymorphism K262R in CYP2B6 has been associated with a higher clearance of bupropion, though its effect on other CYP2B6 substrates remains to be evaluated. In this study, we investigated whether four genetic variants of the CYP2B6 gene (R22C, Q172H, K262R and R487C) were associated with nicotine and cotinine plasma pharmacokinetic measures in 212 healthy Caucasian volunteers (mean age 41.3 years, 72.2% female) in an in vivo nicotine and cotinine metabolism study of adult twins. We observed that the variant genotype at CYP2B6 K262R was associated with significantly higher nicotine clearance (R/R>R/K>K/K, $p<0.008$). Furthermore, compared with 262 K/K subjects, individuals carrying one or two variant 262R allele were also associated with significantly higher cotinine clearance ($p<0.005$) and lower cotinine half-time ($p<0.007$). These differences remained significant after adjustment for non-independence between twins in a pair using a bootstrap procedure. This is the first investigation of CYP2B6 genotype and nicotine metabolism in vivo. Our results provide evidence that the CYP2B6 enzyme plays a role in nicotine and cotinine metabolism and suggest that variants at this gene should be further investigated for their potential role in mediating individual susceptibility to nicotine dependence and tobacco-related diseases. Research supported by National Institute of Drug Abuse grant DA016382. CORRESPONDING AUTHOR: Huijun Z. Ring, Ph.D., Center for Health Sciences, SRI International, 333 Ravenswood Ave., Menlo Park, CA 94025; huijun.ring@sri.com.

CYP1A2*1F allele predicts risk of liver toxicity in children prescribed acetaminophen following surgery. *L.J. Sheffield¹, J.M. Wood¹, L.P. James², J. Roche¹, G.A. Chalkiadis¹, D.J. Venter¹, J.J. Pitt¹, A.J. Davidson¹, N.E. Cranswick¹.* 1) Genetic Health Services Victoria, Intensive Care, Anaesthetics, Cancer Genomics, Clinical Pharmacology, Murdoch Childrens Research Institute, University of Melbourne, Melbourne, Victoria, Australia; 2) Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR.

Acetaminophen, (paracetamol), is used extremely widely as an analgesic in the community. We show that CYP1A2 alleles predict if there is a risk of liver toxicity on exposure to therapeutic dosages of paracetamol. Overdose of acetaminophen is a well-known cause of liver failure due to hepato-cellular necrosis. Case reports have suggested liver toxicity may occur in some children who have been given therapeutic dosages of acetaminophen. We report a pharmacogenetic study of 95 children given paracetamol for pain relief following surgery. Hepatic transaminases were measured as was the level of 3-(cystein-S-yl)-acetaminophen protein adducts (3-Cys-A). 3-Cys-A adducts are found in the liver and serum of laboratory animals treated with toxic doses of acetaminophen and are a highly specific biomarker of acetaminophen toxicity. The relationship between occult liver toxicity and the individuals genotype was investigated by genotyping for functional polymorphisms of the three CYP450 genes known to be involved in the oxidation of acetaminophen to the reactive metabolite, NAPQI. The polymorphisms tested were CYP2E1*5A, CYP2E1*5B, CYP2E1*6 CYP2E1*7A, CYP2E1*7B, CYP2E1*7C, CYP1A2*1F, CYP1A2*6, CYP3A4*, CYP3A4*18. Only the CYP1A2*1F polymorphism, (CYP1A2 gene), correlated with our measures of liver toxicity. For cases with a value for aspartate aminotransferase (AST) >100 there were 16/82 children who had allele A of CYP1A2*1F and 0/26 with allele C (the wild type) ($p=.03$). When those having an increased level of 3-cys-A were included 20/82 had allele A and 0/26 had allele C ($p=.01$). Children with allele A (polymorphism) were the only ones with measures of liver toxicity, and no toxicity was seen with allele C (wild type). The risk for occult liver damage for patients homozygous for AA was 1 in 3 whilst AC and CC seemed highly protective.

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UDP-Glucuronosyltransferase 2B7 Genotype and Cotinine Glucuronidation. *A. Valdes¹, D. Katsuyoshi¹, P. Jacob III², N.L. Benowitz², G.E. Swan¹, H. Ring¹.* 1) SRI International, Menlo Park, CA; 2) University of California, San Francisco.

Glucuronidation plays a major role in the detoxification of many tobacco toxins and is carried out by UDP-glucuronosyltransferases (UGTs). Cotinine is a major constituent of cigarette smoke. In this study, we investigated the relationship between the UGT2B7 His268Val polymorphism and rate of cotinine glucuronidation in urine in 246 Caucasian and Hispanic participants (mean age 41.9 years, 61.9% female) in an in vivo nicotine and cotinine metabolism study of adult twins. We found that Tyr-Tyr homozygotes at UGT2B7 position 268 had a lower cotinine glucuronide to total cotinine ratio (0.210.01) than UGT2B7-268 His carriers (0.260.01, $p < 0.003$). The difference remained statistically significant after taking into account the non-independence between twins in a pair using a bootstrap procedure. This is the first investigation of UGT2B7 genotype and cotinine glucuronidation in vivo. Our result is consistent with published data indicating a significantly lower glucuronidating rate among UGT2B7-268 Tyr-Tyr homozygotes for other tobacco-specific metabolites (NNAL) in liver microsomes. A reduction in the rate of glucuronidation may cause the accumulation of tobacco toxins in the body and lead to an elevated risk of cancer. Additional studies are needed to further examine the potential role of UGT2B7 genotype in susceptibility to tobacco-related cancers. Research supported by University of California - Tobacco-Related Disease Research Program grant 12KT-0234. CORRESPONDING AUTHOR: Huijun Z. Ring, Center for Health Sciences, SRI International, 333 Ravenswood Ave., Menlo Park, CA 94025; huijun.ring@sri.com.

Effect of genetic polymorphisms in γ -glutamyl carboxylase (GGCX) and microsomal epoxide hydrolase (EPHX1) on vitamin K cycle activity in warfarin-treated patients. *M. Vecsler^{1,2}, E. Gak^{1,2}, S. Almog^{1,2}, R. Loebstein¹, D. Kurnik¹, N. Austerweil^{1,2}, B. Goldman^{1,2}, H. Halkin^{1,2}.* 1) Danek Gertner Institute of Human Genetics and Institute of Clinical Pharmacology, Sheba Medical Center, Tel Hashomer; 2) Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv; Israel.

Background: GGCX is responsible for post-translational activation of vitamin K-dependent proteins required for normal hemostasis. GGCX activity is dependent on efficient regeneration of reduced vitamin K by the warfarin-sensitive vitamin K epoxide (KO) reductase (VKOR) complex, in the vitamin K redox cycle. This study investigated the influence of genetic polymorphisms in candidate genes on vitamin K cycle activity and its interrelated sensitivity to warfarin.

Patients & Methods: In 100 patients on warfarin at therapeutic steady state, we analyzed candidate polymorphisms in the genes: *GGCX* (G8762A); two putative components of VKOR complex, *EPHX1* (T612C) and glutathione S-transferase A1 (*GSTA1* T-567G and T-631G), as well as the major warfarin-metabolizing enzyme, *CYP2C9* (*CYP2C9**2 and *CYP2C9**3), and determined their effect on vitamin K cycle activity, expressed as KO/plasma warfarin concentration ratio (KO/W) and on the warfarin sensitivity index (WSI), expressed as the INR/plasma warfarin.

Results: Vitamin K cycle activity was significantly associated with age and *CYP2C9*, *GGCX* and *EPHX1* genotypes (multiple $r=0.53$). *CYP2C9* and *GGCX* showed opposite effects on vitamin K cycle activity. Patients with *GGCX* 8762A containing genotypes presented higher KO/W ratio compared to the G/G wild type genotype (9.211.0 vs. 3.44.6, $p=0.02$). *CYP2C9**2 and *3 containing genotypes were associated with lower cycle activity than the wild type genotypes (2.32.5 vs. 3.44.6, $p=0.01$). WSI was independently and significantly associated with *CYP2C9* and *GGCX* genotype (partial $r=0.30$ and -0.34 , respectively).

Conclusion: These results suggest that common genetic variations significantly affect vitamin K cycle activity and that individual combined genotypes of *GGCX*, *EPHX1* and *CYP2C9* could be considered in the prediction of individual sensitivity to warfarin.

Genetic polymorphisms in microsomal epoxide hydrolase (mEH) component of the vitamin K epoxide reductase (VKOR) complex affect warfarin dose requirements. *R. Loebstein¹, M. Vecsler^{2,3}, E. Gak^{2,3}, N. Austerweil^{1,3}, D. Kurnik¹, B. Goldman^{2,3}, H. Halkin^{1,3}, S. Almog^{2,3}.* 1) Institute of Clinical Pharmacology; 2) Danek Gertner Inst Human Genetics, Sheba Medical Center, Tel Hashomer; 3) Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv; Israel.

Background: The dose-response relationship of warfarin is only partially explained by known polymorphisms in the cytochrome P450 2C9 (2C9) gene, responsible for metabolic clearance of the active enantiomer S-warfarin, as well as by age and body weight. We determined the relative influence on warfarin doses of candidate genes of vitamin K redox cycle: mEH and glutathione-S-transferase A1 putative components of the VKOR, and -glutamyl carboxylase (GGCX) gene.

Patients and Methods: We studied the effects of 2C9, mEH, GST and GGCX genotypes, and of vitamin K1 and K epoxide concentrations, on warfarin doses in 100 patients, at stable anticoagulation.

Results: Allele frequencies for 2C9*1, *2 and *3, mEH T₆₁₂C and GGCX G₈₇₆₂A were 76.5%, 12.5% and 11%, 75% and 25% and 70.5% and 29.5% respectively. Warfarin doses differed among the 2C9 (2C9*1, 2C9*2 and 2C9*3) genotypes: 6.3, 5.3 and 3.8 mg/day respectively (p<0.01), with no differences in the other genotypes. Two way analysis of variance examining the effect of 2C9 and each of the candidate genes revealed only mEH to have an effect on warfarin doses: among the 62 patients with 2C9*1 higher warfarin doses were required among the variant homozygotes for the mEH gene 7.52.9 mg/day compared to the heterozygotes 6.54.2 mg/day and the mEH wild type patients 6.02.6 mg/day (p=0.03). Multiple regression demonstrated only age and 2C9 genotype to be associated with warfarin dose requirements with no effect of concurrent medications or mEH genotype. However, logistic regression analysis showed an odds ratio of 3.14 (95% CI: 1.47-6.67) for requiring 7 mg of warfarin/day in the variant mEH patients compared to the wild type.

Conclusions: Beyond the effects of 2C9 genotype, age and weight, variants of the mEH genotype appear to predict warfarin doses 50 mg/week.

Quantitative measurements of succinylacetone in amniotic fluid and plasma using GC/MS: a sensitive approach to prenatal diagnosis and follow up of tyrosinemia type I patients. *D. Cyr¹, G. Villain², R. Giguere¹, C. Auray-Blais¹, B. Lemieux¹, R. Drouin¹.* 1) Medical Genetics, CHUS, Sherbrooke, Quebec, Canada; 2) Biology department, Lille Sciences and technologies University, France.

Tyrosinemia is a genetic disorder caused by a reduced activity of fumarylacetoacetate hydrolase. Succinylacetone (SA), the diagnostic metabolite, derives from the accumulation of fumarylacetoacetate, a toxic molecule. In untreated patients, SA can easily be measured in plasma and urine because the expected concentrations are in the $\mu\text{mol/L}$ range. Due to a founder effect, the province of Quebec has an unusually high prevalence of tyrosinemia cases. Therefore, the quantification of SA in the nmol/L range in amniotic fluid (AF) or blood of patients treated with NTBC becomes very useful. This goal can be attained with a GC/MS assay, using a stable isotope dilution process and a newly and more sensitive electronic impact MS technology. The method utilizes 5- C^{13} -SA as an internal standard (IS) and a three steps sample treatment consisting of an oximation (to protect the keto groups), a liquid-liquid extraction and a tri-methylsilyl derivatization. After a 3l GC splitless injection, we validated the method by recording the intensities of the ions m/z 620 for SA and m/z 625 for the IS to demonstrate the specificity, recovery, limit of quantification and detection, accuracy, metabolite stability and precision of measurements. Our experience suggests that samples must be kept at -80C for a storage longer than a week. The mean overall recoveries of SA, using the liquid-liquid extraction procedure, is 90% 6%, the coefficient of variation is 6% and the limit of detection is 1 nmol/L . Control values in AF revealed a mean of 4 nmol/L (range: not detected (nd)-12 nmol/L); in plasma, a mean of 3 nmol/L (range: nd-6 nmol/L). The utility of this method was demonstrated by quantification of SA in the AF sample of an affected foetus (SA=2102 nmol/L) and in plasma from patients treated with NTBC (mean=26 nmol/L , range: 16-44 nmol/L , n=19). These preliminary results demonstrate that this method is suitable for clinical use and will be enhanced by further urinary testing and inter-laboratory comparative studies.

Conversion of the substrate preference of isovaleryl-CoA dehydrogenase to 2-methylbutyryl-CoA by site-directed mutagenesis. *E. Goetzman, A. Mohsen, K. Prasad, J. Vockley.* Children's Hospital of Pittsburgh, University of Pittsburgh School of Medicine.

The amino acid residues that line the substrate binding pocket of isovaleryl-CoA dehydrogenase (IVD) are conserved in over twenty known and putative IVDs found in GenBank with the exception of an IVD-like sequence from the potato plant (St-IVD-1) that differs at five key residues in the substrate binding pocket. Potato plants possess a second IVD gene (St-IVD-2) with the usual amino acid sequence in the binding pocket. Both proteins are 63% identical to human IVD. Both were expressed in *E. coli* and purified. Recombinant St-IVD-2 showed maximal dehydrogenase activity with isovaleryl-CoA as substrate (K_m of 1.3 M). In contrast, St-IVD-1 was maximally active with 2-methylbutyryl-CoA (K_m of 1.0 M), but had no activity with isovaleryl-CoA. Isovaleryl-CoA and 2-methylbutyryl-CoA are five-carbon branched-chain substrates that differ in the position of the branch. In mammals they are catabolized by separate enzymes, IVD and short/branched-chain acyl-CoA dehydrogenase (SBCAD), which are only 35% homologous. We hypothesized that the five differing residues in the substrate pocket of St-IVD-1 were sufficient to change the preferred substrate from isovaleryl-CoA to 2-methylbutyryl-CoA. Human IVD was mutated to match St-IVD-1 at these five residues and expressed in *E. coli*. The five mutations abolished activity toward isovaleryl-CoA, but the recombinant enzyme could now utilize 2-methylbutyryl-CoA. Thus, a minimal number of substitutions are sufficient to convert human IVD into an SBCAD. Three of these residues (at positions 95, 99, and 103) lie in helix E of human IVD, which forms one side of the substrate pocket, while the other two residues (370 and 374) are located across the pocket near the catalytic base. A mutant human IVD containing only the three helix E mutations was inactive with both substrates while the substrate specificity of a L370M/G374A double mutant was similar to wild-type IVD. These data suggest the three helix E substitutions play the major role in conferring the 2-methylbutyryl-CoA dehydrogenase activity to St-IVD-1.

A viable knock-in mouse model of congenital erythropoietic porphyria. *D.F. Bishop, M.C. Ramirez, A. Shady, R.J. Desnick.* Department of Human Genetics, Mount Sinai School of Medicine, New York, NY.

Congenital erythropoietic porphyria (CEP) results from the markedly deficient, but not absent, activity of the fourth enzyme in the heme biosynthetic pathway, uroporphyrinogen III synthase (UROS). The enzymatic defect leads to the accumulation of uroporphyrin (URO) and coproporphyrin (COPRO) I isomers in erythrocytes, plasma, urine, and feces. Based on the amount of residual activity, the disease phenotype varies from non-immune hydrops fetalis to milder, later-onset cutaneous involvement. A murine model would facilitate the evaluation of various therapeutic strategies, however, the homozygous UROS knockout was an early fetal lethal. Thus, we generated knock-in mice for two UROS missense mutations, C73R and V99A, that result in low enzymatic activity, the purified murine recombinant C73R and V99A enzymes having 0.1 and 2% of wild type enzymatic activity, respectively. These mutations were individually introduced into an ES cell targeting vector containing an 11 kb murine UROS genomic sequence with adjacent silent restriction sites and a *neo* selectable marker. After homologous recombination in SV129 ES cells, germline transmission of both mutations was obtained. The homozygous C73R mouse was an early embryonic lethal, whereas the V99A homozygotes and heteroallelic C73R/V99A mice were viable and fertile. Both V99A/V99A and C73R/V99A mice exhibited prominent erythrodontia, providing rapid identification. Compared to wild-type mice, the fecal URO I and COPRO I isomers were elevated in the porphyric mice 9 to 12-fold and 68 to 84-fold, respectively. In the V99A/V99A mouse, plasma URO I was elevated 6-fold over wildtype, while in the V99A/C73R mouse, plasma URO I increased 34 fold, consistent with the lower residual activity of the heteroallelic CEP mouse. Hematologic indices were normal for both lines and the development of cutaneous lesions is being evaluated. Thus, these mice have the biochemical and clinical phenotype of CEP and provide a model for the evaluation of therapeutic gene and stem cell strategies.

A case of Fanconi Bickel syndrome caused by uniparental disomy of chromosome 3 with hyperinsulinism and hyperammonemia. *T.L. Hoffman¹, E. Blanco⁵, A. Lane⁵, P.G. Parton⁵, D. De León², I. Gadi⁴, I. Krantz¹, R. Santer³, C. Stanley², T. Wilson⁵.* 1) Pediatric Genetics, Children's Hospital of Phila, Philadelphia, PA; 2) Pediatric Endocrinology, Children's Hospital of Phila, Philadelphia, PA; 3) University Childrens' Hospital, Hamburg, Germany; 4) Department of Cytogenetics, Labcorp, Research Triangle Park, NC; 5) Pediatric Endocrinology, State University of New York, Stony Brook, NY.

Fanconi Bickel syndrome (FBS) is caused by mutations in the facilitative glucose transporter GLUT2. Patients with FBS present with hypoglycemia, hepatomegaly, failure to thrive, and renal Fanconi syndrome with vitamin D-resistant rickets. We describe a 2 year old female with FBS characterized by a more severe phenotype than previous reports. Our patient suffered from severe hypoglycemic seizures and required high glucose infusion rate from intravenous dextrose infusion to maintain normoglycemia. She had significant developmental delays with absent speech. Lab analysis indicated hyperinsulinism and mild hyperammonemia, two atypical features not previously seen in FBS. Treatment with diazoxide allowed discontinuation of intravenous dextrose and reduction of glucose infusion rate such that enteral feeds could maintain normoglycemia. Sequencing of genomic DNA indicated that the child was homozygous for a previously described null mutation in GLUT2 (C1213T). Molecular genotyping of the child's parents showed that only the mother was a carrier of the C1213T mutation. Microsatellite marker analysis confirmed paternity and indicated that there was homozygous inheritance of the C1213T allele in the child via maternal isodisomy of chromosome 3. Uniparental disomy (UPD) of chromosome 3 has never been described and represents a novel mode of transmission for FBS. The implications of UPD 3 on the severity of the child's phenotype are discussed.

X-Inactivation studies in Fabry female carriers. *A. Morrone¹, C. Cavicchi¹, L. Carraresi¹, T. Bardelli¹, M.A. Donati¹, D. Antuzzi², R. Parini³, S. Feriozzi⁴, R. Barone⁵, G. Pistone⁶, R. Di Vito⁷, A. Salviati⁸, R. Ricci², E. Zammarchi¹.* 1) Dept of Pediatrics, Azienda Osp Meyer, Univ of Florence, Florence, Italy; 2) Catholic Univ, Rome; 3) S Gerardo Hospital, Monza; 4) Belcolle Hospital, Viterbo; 5) Dept of Pediatrics, Catania; 6) Dept of Dermatology, Palermo; 7) Dept of Nephrology, Chieti; 8) Dept of Neurology, Verona.

Fabry disease is an X-linked lysosomal storage disorder caused by a primary deficiency of α -galactosidase A. Female carriers can be asymptomatic or clinically affected. Because of X-chromosome inactivation, heterozygous females can show normal GLA activity and mutation analysis is the only certain method of detecting female carrier status. We identified GLA gene mutations in 40 females from 21 unrelated families with a positive family history of Fabry disease. Up to now very few studies on X-inactivation have been carried out on Fabry carriers. In order to determine an eventual correlation with clinical manifestations in Fabry carriers, X-inactivation studies were carried out on the peripheral blood DNA of 35 female Fabry carriers. Of these 27 showed clinical signs or symptoms and were classified as manifesting carriers (MC), while 8, with no clinical sign or symptom, were defined as asymptomatic (A). 13 (11 MC and 2 A) showed a random X-inactivation pattern. 16 MC and 6 A, showed a moderately skewed and/or skewed X-inactivation pattern: 14 MC in favour of the mutant allele, 6 A in favour of the wild type allele and 2 MC in favour of the wild type allele but in the latter a possible recombination cannot be excluded. These X-inactivation studies suggest that female carriers with a random, moderately skewed and /or skewed X-inactivation pattern in favour of mutant allele have an increased risk of developing symptoms ($p < 0.001$), while carriers showing a moderately and/or skewed pattern in favour of wild type allele are more likely to be asymptomatic ($p < 0.001$). X-inactivation studies and accurate clinical evaluations could be helpful in predicting the female phenotypes and give useful indications for the therapeutic management of manifesting carriers. TKT Europe 5S and Genzyme Corporation are gratefully acknowledged.

Increased blood brain barrier permeability caused by loss of function of thymidine phosphorylase in patients with MNGIE. *K. Szigeti¹, N. Sule², A.M. Adesina², D.L. Armstrong², G.M. Saifi¹, E. Bonilla³, M. Hirano³, J.R. Lupski¹.* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Dept pathology, Baylor Col Medicine, Houston, TX; 3) Dept of Neurology, Columbia Univ Col of Physicians and Surgeons, New York, NY.

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is an autosomal recessive multisystemic disorder caused by thymidine phosphorylase (TP) deficiency. While the pathomechanism of the secondary mitochondrial dysfunction has been extensively studied, that of the leukoencephalopathy has not been elucidated. We hypothesized that the white matter hyperintensities on T2 weighted MRI images reflect disturbance of blood-brain barrier (BBB) function. We performed immunohistochemistry (IHC) for TP and intracellular albumin in 2 postmortem brain specimens from patients affected with MNGIE and compared them to normal age-matched controls (N=4) and to a patient with FK 506-induced posterior reversible leukoencephalopathy (PRLE), a disease caused by BBB breakdown. TP is expressed in astroglia, endothelial cells, microglia and to a variable degree even in cortical neurons in the human telencephalon in contradistinction to rodents. Albumin IHC disclosed quantitative ($p < 0.01$) and qualitative differences between the MNGIE and control brains. These data indicate that loss of TP function impairs the integrity of the BBB. MNGIE provides a unique opportunity to examine the effects of loss-of-function of TP in the human central nervous system.

Mapping of a new autosomal recessive non-syndromic hearing loss locus to chromosome 5q13. *L. Basel-Vanagaite*^{1,2}, *R. Atia*³, *N. Magal*³, *T. Shohat*^{4,5}, *E. Taub*¹, *M. Shohat*^{1,2}. 1) Dept Medical Genetics, Rabin Medical Ctr, Petah Tikva, Israel; 2) Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel; 3) Felsenstein Medical Research Center, Petah Tikva, Israel; 4) Israel Center for Disease Control, Israel Ministry of Health, Tel Hashomer; 5) Department of Epidemiology and Preventive Medicine, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel.

Isolated deafness is a genetically heterogeneous trait. Approximately 80% of genetic hearing loss is non-syndromic. The recessive mode of transmission accounts for approximately 75% of inherited cases of non-syndromic deafness. We have ascertained 5 individuals from a large consanguineous family with profound congenital autosomal recessive deafness. All the affected individuals are otherwise healthy. Following a genome wide screening, linkage was detected to locus D5S424 on chromosome 5, thereby defining a novel deafness locus. Two-point linkage analysis resulted in a maximum lod score of 3.54 ($\theta = 0.00$) for marker D5S424. Recombination events defined a 15 Mb critical region between GATA141B10 and D5S428. Fine mapping and identification of candidate genes are in progress.

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GERON Genotyping a centralized data management and analysis system for genetic linkage and association studies. *S. Bandyopadhyay, J.R. Gibbs, D. Hernandez, J.F. Duckworth, A. Singleton.* Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Building 10, Room 6C103, MSC1589, Bethesda, MD 20892, USA.

The GERON (GEnetics Research ONline) Genotyping data management system provides a repository for the storage and mining of genotype data as well as streamlining the multitude of steps required to perform any type of marker based linkage/association analysis. The system has the capacity to handle polymorphic marker data for family and case-control based genetics research. The database includes current Genethon allele frequency data, fragment sizes for 891 CEPH (Centre d'Etude du Polymorphisme Humain) reference individuals at 10251 unique markers and also enables the user to build their own estimates of allele frequencies based on analysis of ethnicity specific genotyping. The database automatically applies allele offsets based on CEPH genotypes. The database includes dynamic links to UniSTS and dbSNP for marker information, and integrates genetic location data from several different published genetic maps. The system has the ability diagnose and track mendelian errors, perform simple statistical analysis and export to various linkage/association analysis file formats. GERON is available from <http://neurogenetics.nia.nih.gov>.

Haplotypic association spanning the 22q11.21 genes COMT and ARVCF with schizophrenia. *M. Martinez*¹, *A.R. Sanders*², *I. Rusu*², *J. Duan*², *J.E. Vander Molen*³, *C. Hou*², *S.G. Schwab*^{4,5,6}, *D.B. Wildenauer*^{4,7}, *P.V. Gejman*². 1) EMI 00-06, E0006/15932, INSERM EMI 00-06, Evry, France; 2) Center for Psychiatric Genetics, Evanston Northwestern Healthcare Research Institute, and Northwestern University, Evanston, IL; 3) Department of Human Genetics, University of Chicago, Chicago, IL; 4) School of Psychiatry and Clinical Neurosciences, University of Western Australia (UWA), Perth, Australia; 5) Centre for Medical Research, UWA, Perth, Australia; 6) Department of Psychiatry, University of Bonn, Bonn, Germany; 7) Centre for Clinical Research in Neurosciences, UWA, Perth, Australia.

Catechol-O-methyltransferase (COMT) has been implicated in schizophrenia by its function through its roles in monoamine neurotransmitter metabolism and its impact on prefrontal cognition, and also by its position through linkage scans and a strong cytogenetic association. Further support comes from association studies, especially family based ones examining the COMT variant, Val^{108/158}Met. We have studied eight markers spanning COMT and including portions of the two immediately adjacent genes, thioredoxin reductase 2 (TXNRD2) and armadillo repeat deleted in velocardiofacial syndrome (ARVCF), using association testing in 136 schizophrenia families. We found nominal evidence for association of illness to rs165849 ($P=0.051$) in ARVCF, and a stronger signal (global $P=0.0019-0.0036$) from three-marker haplotypes spanning the 3 portions of COMT and ARVCF, including Val^{108/158}Met with Val^{108/158} being the over-transmitted allele, consistent with previous studies. We also find Val^{108/158}Met to be in linkage disequilibrium with the markers in ARVCF. These findings support previous association signals of schizophrenia to COMT markers, and suggest that ARVCF might contribute to this signal. ARVCF, a member of the catenin family, besides being a positional candidate, is also one due to its function, i.e., its potential role in neurodevelopment, which is implicated in schizophrenia pathogenesis by several lines of evidence.

Model-independent linkage analysis and association tests in a group of families with kyphoscoliosis: a comparison between microsatellites and single-nucleotide polymorphisms. *C.M. Justice¹, M.H. Roy-Gagnon¹, B. Marosy², S. Novak², P. Boyce³, J. Pettengill³, K.F. Doheny³, E.W. Pugh³, N.H. Miller², A.F. Wilson¹.* 1) Genometrics Section, NHGRI/NIH, Baltimore, MD; 2) Department of Orthopaedic Surgery, JHU, Baltimore, MD; 3) CIDR, IGM, JHU SOM, Baltimore, MD.

Kyphoscoliosis is an unusual deformity in which a lateral curvature of the spine is seen in conjunction with thoracic hyperkyphosis in otherwise normal individuals. As part of a large collaborative study of familial idiopathic scoliosis, 202 families (1198 individuals) with 2 or more individuals with a lateral curvature ≥ 10 were ascertained and clinically characterized. Seven families (53 individuals) had at least two individuals with kyphoscoliosis (lateral curvature ≥ 10 and thoracic kyphosis >40) documented prior to skeletal maturity. The average degree of lateral curvature for individuals with and without kyphoscoliosis was 48 and 31, respectively. The average degree of kyphosis was 53. A genome-wide screen was performed at the Center for Inherited Disease Research using a modified CHLC v.9 marker set of 391 short tandem repeat polymorphisms (STRPs). Individuals with kyphoscoliosis or scoliosis were classified as affected. Sib-pair analysis suggested linkage to chromosomes 2, 5, 13 and 17. An additional 25 STRPs were genotyped in the candidate regions. The most significant p-values were obtained for chromosomes 5 and 13. To narrow further the candidate regions, 71 SNPs were genotyped in a ~ 10 Mb region of 5p13 using a mass spectrometer. Results were read using SpectroTYPER software (Sequenom). Using a candidate region approach, 145 SNPs (14 candidate genes) on chromosome 13 and 13 SNPs (1 candidate gene) on chromosome 5 were genotyped with SNPs from ABI and read with the Perkin-Elmer ABI Prism 7700 Sequence Detection System. Additional SNPs were genotyped under the Illumina platform. One panel was genotyped for chromosome 5 (.05 Mb density) and another panel for chromosome 13 (.131 Mb density). Results are presented (graphically) and compared for sib-pair linkage analysis and tests of association using only the STRPs, only the SNPs, and the STRPs and SNPs combined for each platform and all platforms combined.

The a-Catenin polymorphisms associated with amyloid levels, are not a strong risk factor for late onset

Alzheimers disease. *D. Avramopoulos¹, M.D. Fallin², S.S. Bassett¹*. 1) Dept. of Psychiatry, School of Medicine, Johns Hopkins Univ, Baltimore, MD; 2) Dept. of Epidemiology, School of Public Health, Johns Hopkins Univ, Baltimore, MD.

A recent study by Ertekin-Taner et al detected a strong association between intronic polymorphisms in the a-Catenin gene (VR22) and the levels of beta Amyloid (A-beta) in Alzheimers disease patients, thus implicating VR22 in the pathogenesis of late onset Alzheimers disease. That study was a follow up to a strong linkage finding by the same group for a quantitative trait locus for A-beta on chromosome 10q, a candidate gene approach that examined VR22 because of its location and functional relevance. We recently reported linkage for Late Onset Alzheimers Disease (LOAD) on chromosome 10q in families of maternal disease origin from the NIMH genetics initiative pedigrees, in a region that overlaps with the QTL described above. Although VR22 lies just outside our linkage interval, we decided to test whether this gene is also a risk factor for LOAD, and whether the presence of the high risk allele is correlated with the observed linkage signal in our pedigrees. We genotyped the two SNPs showing the strongest effect in the Ertekin-Taner study for 182 of the NIMH genetics initiative pedigrees, those that include both affected and older unaffected members. Our analysis did not detect an association or trend. We then genotyped the remaining NIMH pedigrees in order to examine the correlation between the presence of the high A-beta load associated allele and linkage at VR22 using the GIST method and software. No such correlation was detected. Given the failure of two independent tests for the effect of these polymorphisms we conclude that despite their strong association with A-beta loading, they do not appear to have an equally strong effect on the risk for LOAD, and that the gene(s) underlying our linkage signal close to the centromere of chromosome 10 in the NIMH pedigrees remains to be identified.

Mapping of a new locus for autosomal recessive spastic paraplegia. *N. Bouislam^{1,2}, A. Benomar², A. Bouhouche², H. Azzedine¹, A. Durr^{1,3}, A. Brice^{1,3}, M. Yahyaoui², G. Stevanin^{1,3}.* 1) Institut des Neurosciences, INSERM U289-NEB, Paris, France; 2) Laboratoire de Neurogenetique, Hopital des Specialites, Rabat, Morocco; 3) Department of Genetics, Cytogenetics and Embryology APHP, Hopital Salpetriere, Paris, France.

Hereditary spastic paraplegias (HSP) are characterized by progressive spasticity in lower limbs, due to the dysfunction and/or degeneration of the upper motor neuron. It is classical to distinguish between pure and complex HSP. The pure forms present with pyramidal signs (brisk reflexes, Babinski sign, spasticity, motor deficit, +/- sphincter disturbances) often associated with deep sensory loss. Complex forms variably associate numerous combinations of neurological and extra-neurological signs such as ataxia, dysarthria, neuropathy, optic atrophy or retinitis pigmentosa, hearing loss, mental retardation. Molecular studies in HSP have confirmed the heterogeneity at the genetic level and 20 loci have been mapped to date. We have identified a large consanguineous family (11 subjects including 3 affected members) of Moroccan ancestry with pure spastic paraplegia displaying an autosomal recessive pattern of inheritance of the disease. All 3 affected family members had onset of spasticity in the lower limbs between ages 10-15 and required walking aid at ages 26, 38 and 42. A genome wide study suggested linkage to a new locus on chromosome 14. Linkage analysis of 27 microsatellite markers allowed to obtain a maximal multipoint lod score of 3.21. Haplotype reconstruction restricted the candidate region to a 5 cM interval encompassing 10 markers at the homozygous state among the patients. Direct sequencing of the coding exons of two candidate genes was performed but no disease causing mutation was found. This pedigree represents a genetically distinct form of pure and slowly progressive HSP mapped to chromosome 14.

Characterization and homozygosity mapping to 9p24 of a recessive syndrome of nonprogressive cerebellar ataxia and mental retardation in the Hutterite Brethren. *K.M. Boycott¹, J. Parboosingh¹, H.C. Glass², E. Wirrell², J.*

Scott³, K. Davey¹, S. Flavelle¹, A.E. Chudley⁴, A. Bureau¹, D.R. McLeod¹. 1) Dept Medical Genetics, University of Calgary, Calgary, AB, Canada; 2) Dept Pediatrics, University of Calgary, Calgary, AB, Canada; 3) Dept Radiology, University of Calgary, Calgary, AB, Canada; 4) Dept Medical Genetics and Biochemistry, University of Manitoba, Winnipeg, MB, Canada.

The Hutterites are an endogamous German-speaking people that originated from the Anabaptist movement during the 16th century. We reviewed and examined 19 Hutterite patients with developmental delay and previously documented cerebellar signs. Patients completed a standard set of investigations, including magnetic resonance imaging of the brain. Twelve Hutterite patients from six interrelated sibships had clinical presentations consistent with Dysequilibrium syndrome (DES), a syndrome of nonprogressive cerebellar ataxia and mental retardation described in a small number of Hutterite patients in the 1980s. Clinical features of our patient group included mild to profound mental retardation, strabismus, pes planus, walking after age six, relatively marked truncal ataxia with limited peripheral ataxia and a static clinical course. Variable features included short stature and seizures. The underlying structural brain abnormalities identified on neuroimaging of Hutterite DES patients are distinct and include hypoplasia of the inferior portion of the cerebellum with absence of the inferior portion of the vermis, partial formation of the primary fissure and cerebellar hemispheres, small pons, and simplification of the cerebral cortex.

The autosomal recessive inheritance of DES and the endogamous nature of the Hutterite population have facilitated homozygosity mapping studies. A genome scan using 400 microsatellite markers at a density of 10 cM identified a region of homozygosity spanning 17 cM and localized the DES gene between 9ptel and D9S286. Candidate genes are currently being evaluated. Identification and functional analysis of the DES gene will provide new insights into cerebellar development.

Analysis of Multiple Polymorphisms in the Serotonin Transporter (SLC6A4) in Autistic and Control Populations. *I.J. Chacon, A.L. Yonan, R. Cheng, E. Cayanis, J.D. Terwilligwe, A.A. Palmer, T.C. Gilliam.* Columbia Genome Center, Columbia University, NY, NY 10032.

Twin and family studies demonstrate a strong genetic contribution to the development of Autism Spectrum Disorders (ASD), yet no risk alleles have been unambiguously identified. We previously reported a genomewide scan to identify autism susceptibility loci in 345 multiplex families (AGRE). The most significant finding was an MLS of 2.83 on chromosome 17q, which increases to 4.41 among a subset of families with only affected males (Stan Nelson, personal communication) and harbors a strong candidate gene for ASD, the serotonin transporter (5-HTT; SLC6A4). Here, we report a survey of the putative functional promoter polymorphism (5-HTTLPR) together with neighboring SNPs in two large ASD samples (AGRE and Risch et al. 1999) and an independent control sample. We noted an interesting trend wherein the 5-HTTLPR variant and SNPs that map within the GC-rich repeat sequence elements of the promoter were difficult to genotype and violated Hardy-Weinberg (HW) equilibrium in both ASD and control populations. Neighboring SNPs that map outside the promoter region were more reliably genotyped and met HWE. Specifically, HW disequilibrium was detected for 5-HTTLPR in the unaffected founders of both autistic populations as well as the controls and three SNPs that lie within the promoter region did not produce reliable genotypes. By contrast, two SNPs localized in the 5' UTR region (rs2020939 and rs2020936) and a third in intron 2 (rs1872924) show conformation to HWE and point to allelic association to ASD. We detect evidence of preferential transmission of an allele of SNP3 (located 26Kb from the 5-HTT promoter) in the Risch autism sample (FBAT ver-1.4.2; Z-score: 2.10, p-value:0.035), and of a haplotype containing this same allele in a preliminary analysis of 170 AGRE pedigrees added to the Risch sample (FBAT; Z-score: 2.23, p-value: 0.026). We will complete this systematic survey of the 5-HTT polymorphisms in and surrounding the promoter region and discuss potential ramifications of these studies for the genetic analysis of autism and other neuropsychiatric disorders.

Linkage Disequilibrium-Based Investigation of Candidate Genes for Developmental Dyslexia in the Chromosome 6p21.3-22.3 Region. *J. Couto¹, K. Wigg¹, Y. Feng¹, B. Anderson², T. Cate-Carter², R. Tannock², M. Lovett², T. Humphries², C.L. Barr^{1,2}.* 1) The Toronto Western Research Institute, University Health Network, Canada; 2) The Hospital for Sick Children, Toronto, Canada.

Developmental dyslexia or specific reading disability (RD) is a complex cognitive disorder, defined as severe difficulty in reading despite average intelligence and adequate opportunity. It affects 3-10% of children and tends to persist into adulthood. RD is heritable, and linkage to several chromosomal regions has been identified. The most replicated finding to date is for linkage in the 6p21.3-22.3 region on the short arm of chromosome 6, although the susceptibility gene(s) in this region has not yet been identified. The objective of this study was to establish a dense set of markers in 6p21.3-22.3, and to fine-map and identify RD susceptibility genes. We investigated 45 SNPs (single nucleotide polymorphisms), in a sample of 180 nuclear families ascertained through children with reading difficulties at school. Diagnosis of RD, as a categorical trait, was made using 3 core tests that assess single-word reading and phonological decoding. Additional quantitative reading and related phenotypes were assessed using tests for the partially overlapping skills that contribute to reading ability. Candidate genes included the genes for cAMP response element like protein 1 (CREBL1) and cyclin dependent kinase 5 (CDK5) regulatory subunit associated protein 1-like 1 (CDKAL1). Using the transmission disequilibrium test, we found significant evidence for association of categorically defined RD to two SNPs in the gene for CREBL1 ($p=0.014$; $p=0.040$) and one SNP in CDKAL1 ($p=0.018$) in the 6p region. Evidence for association was also found to another polymorphism in CDKAL1 for phonological decoding, a quantitative phenotype of RD ($p=0.044$). The two SNPs in each gene are in linkage disequilibrium (LD) with each other, $D=0.884$ and 1.000 respectively. These two positive regions are 10.9 Mb apart and are not in LD with each other. It is likely that one region is an artifact of limited statistical power. We will be able to distinguish between the two as our sample size increases, and we further investigate both regions.

Genetics of autism and the broader phenotype in Iceland: Genome scan for autism and ASD. *R. Fossdal¹, E. Saemundsen², G. Bjornsdottir¹, P. Magnusson³, B. Lauth³, S. Sieberts¹, S. Hreidarsson², S. Thorvaldsson¹, O. Gudmundsson³, J. Thorhallsdottir¹, H. Palsdottir¹, B. Unnarsdottir¹, J. Gulcher¹, H. Stefansson¹, K. Kristjansson¹, T.E. Thorgeirsson¹, K. Stefansson¹.* 1) CNS, deCODE Genetics, Inc., Reykjavik, Iceland; 2) State Diagnostic Counseling Center, Reykjavik Iceland; 3) Department of Child and Adolescent Psychiatry, National University Hospital, Reykjavik Iceland.

The autism spectrum includes a few syndromes with variable severity and age at onset for impaired development of social skills, repetitive behaviors and verbal and non-verbal communication problems. Although autism has a striking genetic component according to twin studies, a number of genome scans and association studies performed in the last decade have not yet led to the isolation of definite autism genes. Additional studies are therefore needed. We have begun a study of the genetics of autism and autism spectrum disorders (ASD) in Iceland that is based on linkage within large extended families. Here we describe the family cohort of 352 individuals with autism or ASD and their relatives. The study includes the systematic phenotypic ascertainment of the unaffected relatives to identify individuals with signs of the broader autism phenotype as judged by their scores on a screening instrument that we have developed, The Development, Social Interaction and Mood Questionnaire (DSIM), and previously described screening instruments (SRS and SCQ). We also describe the genealogical relationships between ASD and psychiatric phenotypes (schizophrenia, BP, depression, and anxiety). The aim of the project is to include the broader autism phenotype in linkage analysis, but here we present the results of the genome scan for autism and ASD probands (170 affected). To ensure a high information content for this scan, all affected individuals, as well as their parents, siblings, and grandparents, have been genotyped with 2000 microsatellite markers. This scan is based on approximately half of the autism-ASD family cohort, and the results provide support for previously published linkage peaks on chromosomes 5p, 15q and Xq. The research is supported by The Simons Foundation.

SNP genomic screening provides further evidence for genetic heterogeneity in essential tremor. *J.R. Gilbert, L. Zhang, S. Knauer, C. Haynes, C. Salzman, M.M. Menold, S. West, J. Stajich, B. Scott, A.E. Ashley-Koch.* Duke University Medical Center, Durham, NC.

We previously conducted a microsatellite genomic screen on a large essential tremor (ET) family (DUK 13001) that was unlinked to published ET loci on chromosomes 2 and 3. Several interesting regions emerged, but because of the moderate size of this pedigree, no conclusive linkage could be established. To aid in the linkage of this family, we conducted a 10K SNP chip genomic screen in this, and 2 other moderately sized ET families (DUK 13041 and DUK 13028). Expected 2pt lod scores for each family, assuming an affecteds only dominant model with a two allele marker, were generated with SIMLINK and were as follows: DUK 13001: 2.0; DUK 13041: 2.3; and DUK 13028: 1.2. Peak 2pt lod scores were obtained for DUK 13001 on chromosomes 3 (lod=1.6), 5 (lod=1.4), 15 (lod=1.6) and 20 (lod=1.4). Regions on all chromosomes except chromosome 3 had also provided positive 2pt lod scores with the microsatellite genomic screen of this family. The region on chromosome 3 did not overlap with the previously reported ET linkage on chromosome 3. For DUK 13041, peak 2pt lod scores were obtained on chromosomes 1 (lod=2.0), 4 (lod=1.7) and 9 (lod=1.7). For DUK 13028, peak 2pt lod scores overlapped regions on chromosome 4 (lod=1.1) with DUK 13028, and chromosome 5 (lod=1.0) with DUK 13041. However, the best evidence for linkage with DUK 13028 was on chromosome 17 with a peak 2pt lod score of 1.5. Because none of the peak regions for families DUK 13001 and DUK 13041 overlap, these data provide further evidence for genetic heterogeneity in ET and suggests that there are additional ET loci that remain as yet unidentified. We are currently characterizing the patterns of LD amongst the SNPs in the genomic screen and performing multipoint analysis to further refine these high-priority regions.

Towards the identification of the anx mutation in the anx/anx murine model for anorexia nervosa. *M. Gratacos, JM. Mercader, M. Ribases, X. Estivill.* Genes and Diseases, Center for Genomic Regulation, Barcelona, Spain.

Anorexia Nervosa (AN) is a complex disorder where both socio-cultural and genetic factors are supposed to be involved. The mutant *anx/anx* is a spontaneous murine model for AN, as mutants are characterized by poor appetite, growth failure, and abnormal behavior such as head weaving and hyperactivity. This model is due to a recessive autosomal mutation, not yet identified, but mapped through linkage analysis close to the Pallidin gene (*Pa*), located at 122,9 Mb in chromosome 2. Several studies on differential gene expression and on neuropeptide distribution have been performed in order to disclose the etiology of this phenotype, but no additional data on the position of the *anx* gene has been published until now. The aim of this study was to map the genetic position of the *anx* gene through the typing of 30 microsatellite markers and covering a region of 30 Mb around the *Pa* gene. The offspring of several experimental crosses of carrier heterozygotes (B6C3Fe-*anx* A/+ a) were analyzed and over 400 meioses are being screened using three multiplex reactions of 10 microsatellites each. Markers were generated from information obtained in databases (DMit markers) and from the available sequence of the mouse genome. Preliminary linkage analysis reveals that the *anx* mutation is located close to position 119 Mb (Tx 119) (lod score 4.5 at a recombination fraction of 0) on mouse chromosome 2. Fine mapping of the *anx* mutation is being performed with SNPs covering the region of synteny between the two parental strains and additional microsatellite markers. Mutation search using dHPLC should facilitate the identification of mutations in candidate genes responsible for the anorexia phenotype in the *anx* model and to identify the mutation causing anorexia, to better understand the mechanisms underlying feeding and body weight regulation, and the genetic factors involved in the development of eating disorders.

A clinical, genetic and neuropathologic analysis of autosomal dominant cerebellar ataxia linked to chromosome 16q22.1 (16q-ADCA type III). *K. Ishikawa¹, S. Toru¹, T. Tsunemi¹, T. Amino¹, H. Fujigasaki¹, M. Sakamoto¹, M. Li¹, H. Tomimitsu¹, K. Owada¹, K. Ishida², M. Gomyoda³, M. Yoshida⁴, Y. Hashizume⁴, I. Kondo⁵, K. Kobayashi⁶, T. Toda⁶, H. Mizusawa¹.* 1) Dept Neurology and Neurological Sciences, Graduate School, Tokyo Medical & Dental Univ, Tokyo, Japan; 2) Dept Neurology, National Matsue Hospital, Matsue, Shimane, Japan; 3) Dept Clinical Laboratory, National Matsue Hospital, Matsue, Shimane, Japan; 4) Dept Neuropathology, Inst Medical Science of Aging, Aichi Medical School, Aichi, Japan; 5) Dept Medical Genetics, Ehime Univ School of Medicine, Onsen-Gun, Ehime, Japan; 6) Dept Post-Genomics and Diseases, Osaka Univ Graduate School of Medicine, Suita, Osaka, Japan.

Autosomal dominant cerebellar ataxia (ADCA) is a group of heterogeneous neurodegenerative diseases showing progressive cerebellar ataxia as a cardinal clinical feature. We mapped families with pure cerebellar ataxia to a 3 cM region in the chromosome 16q22.1 (Nagaoka et al., *Neurology*; 2000). This region was the locus for another ADCA, spinocerebellar ataxia type 4 (SCA4)(Flanigan et al., *Am J Hum Genet*; 1996), which shows prominent peripheral neuropathy. To identify the gene for 16q-ADCA type III, we constructed a physical map of the locus and analyzed haplotypes for newly discovered polymorphic DNA markers in 30 families of 16q-ADCA type III. We have so far narrowed the critical region of the gene into 3.8 megabase (Mb) interval between D16S3043 and D16S3095 in 16q22.1. In addition, we identified specific haplotype that completely segregate with the disease in all families. Clinical features of these families revealed that 16q-ADCA type III has the latest age of onset with benign course compared to any other ADCAs with known gene loci. Neuropathologic analysis on two families revealed that the Purkinje cell undergo characteristic degeneration with increase of presynaptic proteins around atrophic Purkinje cell bodies. Such changes are not seen in any other ADCAs. We conclude that 16q-ADCA type III has distinct clinical and neuropathologic features. Testing the specific haplotype is useful for molecular diagnosis of 16q-ADCA type III.

Replication of Evidence for Modifiers of Age at Onset in Huntington's Disease on 6q23-24 and 18q22: The HD MAPS Study. *J-L. Li¹, V. Wheeler², L. Srinidhi², A. Russ¹, T. Gillis², M. Marcial², L.A. Cupples¹, J.F. Gusella², M.E. MacDonald², R.H. Myers¹, for the investigators of the HD MAPS study.* 1) Department of Neurology, Boston Univ School of Medicine, Boston, MA; 2) Molecular Neurogenetics Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA.

Huntington's Disease (HD) is a fully penetrant, autosomal dominant disorder associated with an abnormal expansion of a stretch of CAG repeats in the first exon of the huntingtin gene. Although repeat size is correlated with the age at onset (AO), and accounts for approximately 70% of the variation in AO, much of this effect is seen in very young onset individuals with very large repeat sizes. AO adjusted for repeat size is strongly heritable suggesting that it has significant genetic modifiers. Our recent whole-genome scan in 629 affected sibling pairs from 295 pedigrees provided suggestive evidence for quantitative trait loci modifying AO at six genomic regions. In the present study, we assessed evidence for linkage in 125 newly recruited sibling pairs from 57 pedigrees (total sample of 352 pedigrees with 754 sibling pairs). Eighteen microsatellite markers were genotyped, three from each of the six linkage loci. We performed a variance components multipoint linkage analysis in the newly recruited and total sample. We also analyzed the subset with CAG repeat size range from 36 to 50 repeats, as the variation in onset age for a given repeat is maximal in this range. Chromosomes 6q23-24 ($p=0.008$), 18q22 ($p=0.05$) showed evidence for linkage in the new sample and in the total sample (LOD=3.74 and 1.41 respectively). Increased evidence for linkage at 18q22 was seen in the group restricted to 36 to 50 repeats (LOD=2.25). These regions contain several interesting candidate genes, encoding proteins with established roles in cell death and nervous system development, including glutamate receptors, apoptosis effectors, transcription factors, and others. Further studies to identify genetic modifiers for AO for HD are underway.

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Permutation linkage analyses identified several novel susceptibility loci for nicotine dependence with the Framingham Heart Study data. *M.D. Li, D. Wang, J.Z. Ma.* Program in Genomics and Bioinformatics on Drug Addiction, Department of Psychiatry, MSC 7792, Univ Texas Health Sci Ctr, San Antonio, TX.

Nicotine dependence is the most prevalent form of drug abuse in the US and throughout the world. Epidemiological studies demonstrated that genetics account for at least 50% of the liability to nicotine dependence. However, there have been very limited linkage studies for this disorder. In this study, we conducted genome-wide permutation linkage analyses on the longitudinal smoking data of the Framingham Heart Study (FHS) to account for the abnormality associated with the smoking phenotype (defined as the number of cigarettes smoked per day). We used empirical thresholds obtained from permutation tests to determine the significance of each genomic region. To minimize environmental effects on linkage analysis results, we organized the longitudinal FHS smoking data from 1970-1988 into four time points. The Haseman-Elston, non-parametric, and variance component methods were used for the analysis. Under the empirical genome-wide thresholds determined specifically for the FHS smoking data, we found four highly significant linkages of nicotine dependence on chromosomes 1, 3, 4, and 13 ($P = 0.001$) and seven significant linkages on chromosomes 7, 8, 9, 11, 16, 17, and 20 ($P < 0.05$). These regions include nine reported previously and two newly detected in this study. These findings strongly indicate that some of these regions may harbor susceptibility loci for nicotine dependence. Further analysis of these positive regions by fine mapping and/or association analysis is warranted. To our knowledge, this study presents the most convincing linkage evidence for nicotine dependence in the field (supported by NIH grant DA-12844).

STRATIFICATION OF AUTISM FAMILIES BASED ON SEX OF AFFECTED CHILDREN IDENTIFIES DISTINCT LINKAGE REGIONS. *B. Merriman¹, J. Stone¹, R. Cantor¹, A. Yonan³, C. Gilliam³, D. Geschwind², S. Nelson¹.* 1) Dept Human Genetics, Univ California, Los Angeles, Los Angeles, CA; 2) Dept Neurology, Univ California, Los Angeles, Los Angeles, CA; 3) Columbia University, New York, New York.

Autism Spectrum Disorder (ASD) is marked by a profound bias in the gender of affected individuals. While the prevalence of ASD is estimated at 1/1000 in the general population (Folstein et al 2001), the ratio of affected males to females is 4-10:1 (Miles et al 2000, Frombonne E. 1999). This male bias is consistent across many studies and is more striking in higher functioning individuals with autism. It has been suggested that to produce an affected female requires a heavier genetic load of susceptibility genes (Tsai et al 1983). In order to test if a more homogeneous genetic group could be identified based on sex of the affecteds, we used genome scan data of Yonan et al (2003), and subdivided affected sibling pair families into two groups based on the sex of their affected children; 138 families contained only affected males while 108 families contained at least one affected female. Reanalysis of the genome scan data indicated two regions of the genome highly skewed due to the sex of affected children. First, there was stronger evidence of linkage to 17q11 (MLS 4.7) in families with affected boys only. The chromosome 17q region has been previously implicated by Yonan, et al. The shift in linkage score by sex-stratification is non-random ($p=0.01$). Second, 4q31-q34 was linked in families with at least one affected girl (MLS 2.8, genome-wide $p<0.01$), a region not implicated in the initial genome scan. Further, in the two sex-split scans we observe a highly non-random enhancement of a total of 16 (mostly minor) linkage peaks, as compared to 42 via simulation results ($p\text{-value} = 10^{-16}$), suggesting the presence of 812 additional linked loci. These results support the notion that the genetic variants which predispose to autism may have profoundly different effects in developing male and female brains. Also, the novel analytical methodology we developed applies to linkage analysis of other diseases with gender biases, and other forms of sample stratification.

Haplotype mapping of a risk locus for multiple sclerosis at the telomeric end of the HLA complex. *J.P. Rubio^{1,2}, R. Burfoot¹, M. Bahlo¹, J. Stankovich¹, L. Johnson^{1,2}, H. Butzkueven², N. Tubridy³, M. Marriott², C. Chapman³, B. Taylor⁵, D. Booth⁴, R. Heard⁴, T.P. Speed¹, G.S. Stewart⁴, T.J. Kipatrick², S.J. Foote¹.* 1) Genetics and Bioinformatics, WEHI, Parkville, VIC, Australia; 2) The Howard Florey Institute of Experimental Physiology and Medicine, Parkville, VIC, Australia; 3) The Royal Melbourne Hospital, Parkville, VIC, Australia; 4) The Westmead Hospital, Westmead, NSW, Australia; 5) The Royal Hobart Hospital, Hobart, TAS, Australia.

We have refined an MS susceptibility locus to a 210 kb genomic interval spanning the class I and extended class I sub-regions of the HLA complex. This work follows an earlier mapping study in which we detected the presence of a susceptibility locus independent of the DRB*1501-DQB1*0602 (DR15) association at the telomeric end of the classical HLA complex. To refine the locus, an additional 34 microsatellite markers (47 in total) spanning the HLA complex (D6S299 to D6S291) were genotyped in an expanded cohort of 166 Tasmanian simplex families and 104 control families. Microsatellite-based haplotypes were analysed using a novel algorithm (HAPLO_CLUSTERS), which was designed in-house. Long-range haplotypic association was observed for a genomic segment spanning 5 Mb from D6S2225 (extended class I) to G51152 (class II), and an 865 kb sub-region (extended class I/class I) was selected for fine-scale mapping with 26 SNPs. Haplotype block structure was observed across the region and a support interval ($p < 0.05$) for haplotypic association was detected for a 201 kb genomic segment encompassing the genes for GABBR1, MOG, HLA-F and HCG4. Ten SNPs mapping to the core risk interval were genotyped in an independent cohort of 356 MS simplex families from the Australian States of Victoria and New South Wales, and significant association was observed for the same risk haplotype. In conclusion, we have employed a systematic fine mapping strategy to localise four candidate genes for MS. Our findings also provide evidence for an additional MS risk locus further telomeric in the extended class I region of the HLA complex.

A Western Canadian family with pure autosomal dominant spastic paraplegia maps to the SPG8 locus. P.N.

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Hereditary spastic paraplegia (HSP) is characterized by a progressive spastic paralysis in the lower limbs. This heterogeneous disorder can exist either in a pure form or a complicated form that is accompanied by other neurological or non-neurological symptoms. *SPG8* HSP is a pure autosomal dominant form of the disease, which has previously been mapped to chromosome 8q23- 8q24 in a Caucasian kindred. Two additional families have been reported, one in Brazil, and another from Britain. Linkage analysis of these families has refined the *SPG8* locus to a 3.4 cM region between markers D8S1804 and D8S1179. We have identified a novel family from British Columbia, Canada, which displays linkage to the *SPG8* locus. Twelve members of this family are affected, nine of whom have had their DNA and clinical information collected. DNA samples have been gathered from ten other family members. Five STS markers were typed spanning from D8S1804 to D8S1128 that formed a haplotype segregating with the disease phenotype. A sixth marker at the upper boundary, D8S586, exhibited a recombination event in an affected family member. Further fine mapping is underway to determine the lower recombinant. The flanking markers currently extend beyond each end of the *SPG8* candidate region; thus the interval cannot be reduced with this family. Clinical features include a spastic gait and lower limb stiffness, typical of pure HSP individuals. The age of onset for affected individuals falls within the fourth and fifth decade. The identification of this large family will help contribute to the elucidation of the *SPG8* gene and provides further evidence for the geographic diversity of HSP *SPG8*.

X-linkage for Parkinsons Disease and Influence of Breakpoints and Recombination Hotspots on Linkage Analysis with Dense Markers: The GenePD Study. *G. Xu¹, A.L. DeStefano¹, J.B. Wilk¹, J. Latourelle¹, R. Prakash¹, J. Maher¹, M. Sun², M.E. MacDonald², J.F. Gusella², R.H. Myers¹, for the GenePD Study.* 1) Dept Neurology, Boston Univ Sch Medicine, Boston, MA; 2) Molecular Neurogenetics Unit, Center for Human Genetic Research, Massachusetts General Hospital, Harvard Medical School Boston, MA.

Parkinson disease (PD), a late-onset neurodegenerative disorder, is characterized by resting tremor, rigidity, slowness of movement and postural instability. Mutations in four genes parkin, -synuclein, DJ-1, and PINK1 have been identified to cause PD in a minority of families and more than ten PD linkage regions have been reported with the susceptibility genes in these regions still under investigation. Two independent studies show PD linkage on the X chromosome. Here we report X chromosome linkage analysis in 308 families (294 affected sib-pairs) from the GenePD Study, which yielded inconsistent multipoint LOD scores using public recombination frequencies. Analysis of 16 micro-satellite markers (STRs) and 24 single nucleotide polymorphisms (SNPs) generated LOD=3.45 at 65cM (42.65 Mb). However, the addition of two STRs and 11 SNPs at the peak region (42.65 Mb to 44 Mb) for a total 18 STRs and 35 SNPs yielded a reduced multipoint LOD=0.81 at 64cM. We explored the source of the discrepancy and found that the 1.2Mb region from 42.4Mb to 43.6Mb of chromosome X is likely a recombination hotspot. The estimation of the genetic distance based on the physical locations of the markers in the region appears to underestimate the recombination frequency. Underestimating genetic distance with dense markers may bias the linkage analysis, resulting in decreased LOD scores. With the advent of genome wide SNP typing for linkage analysis, it will be important to establish accurate genetic distances between markers in close physical proximity and to pinpoint recombination hotspots. We suggest that linkage analysis with SNP genotypes may be more susceptible to map misspecification than that with traditional STR markers, resulting in inaccurate and reduced LOD scores. This study provides evidence that chromosome Xp11 may be implicated in the susceptibility to PD.

Genome linkage screen for prostate cancer susceptibility loci: a comparison of microsatellites versus single nucleotide polymorphisms. *S.N. Thibodeau¹, J.C. Guenther¹, G.B. Christensen¹, S. Hebring¹, C. Rosenow², C.A. Hilker¹, S.K. McDonnell¹, J.M. Cunningham¹, S.L. Slager¹, D.J. Schaid¹.* 1) Dept Lab Med & Pathology, Mayo Clinic, Rochester, MN; 2) Affymetrix, Inc., Santa Clara, CA.

Prostate cancer is one of the most common cancers among men and has long been recognized to occur in familial clusters. Brothers and sons of affected men have a 2-3 fold increased risk of developing prostate cancer. However, identification of genetic susceptibility loci for prostate cancer has been extremely difficult. Although suggestion for linkage has been reported for many chromosomes, most promising regions have been difficult to replicate. In this study, we compare genome linkage scans conducted by microsatellites versus single nucleotide polymorphisms (SNPs) measured in 467 men with prostate cancer, from 167 families. For the microsatellites, the ABI Prism Linkage Mapping Set version 2 with 402 microsatellite markers was used, and for the SNPs, the Early Access Affymetrix Mapping 10K array was used. Our results show that the presence of linkage disequilibrium (LD) among SNPs can lead to inflated lod scores, and this seems to be an artifact due to the assumption of linkage equilibrium that is required by the current genetic linkage software. After excluding SNPs with high-LD, we found a number of new lod score peaks with values of at least 2.0 that were not found by the microsatellite markers: chromosome 8, with a maximum model-free lod score of 2.2; chromosome 2, with a lod score of 2.1; chromosome 6, with a lod score of 4.2; chromosome 12, with a lod score of 3.9. The lod scores for chromosomes 6 and 12 are difficult to interpret, because they occurred only at the extreme ends of the chromosomes. The greatest gain provided by the SNP markers was a large increase in the linkage information content, with an average information content of 61% for the SNPs, versus an average of 41% for the microsatellites. The strengths and weakness of the microsatellite versus SNP markers are illustrated by the results of our genome linkage scans.

An apparently novel cardiomyopathy syndrome maps to a 40Mb pericentromeric region of chromosome X. *M.A. Aldred¹, C.S. Tan¹, M.M. Williams², L.A. Tyfield², R.C. Trembath^{1,3}, I.D. Young³.* 1) Division of Medical Genetics, University of Leicester, Leicester, UK; 2) Dept. of Molecular Genetics, The Lewis Laboratories, Southmead Hospital, Bristol, UK; 3) Leicestershire Clinical Genetics Service, University Hospitals of Leicester NHS Trust, Leicester, UK.

We describe a family in which 3 male children, identical twins and their maternal cousin, were affected by an apparently X-linked syndromic cardiomyopathy. One twin died *in utero* from structural cardiac anomalies. The surviving twin was noted to have an abnormal pattern of cardiac contraction from birth and died in heart failure aged 5 months. Autopsy revealed left ventricular cardiomyopathy, with left ventricular and atrial dilatation and endocardial fibroelastosis. He was also developmentally delayed and facially dysmorphic. The maternal cousin was similarly affected and died aged 6 years. Metabolic and chromosome analyses were normal. The phenotype was not typical for CMD3A or Barth syndrome and mutation analysis of the G4.5 *TAZ* gene was normal. We then examined X-inactivation patterns at the androgen receptor locus in the boys' mothers. Both showed a completely skewed pattern, with the active X-chromosome being that inherited from their unaffected father, thus strengthening the hypothesis of an X-linked trait. In contrast, their mother, also presumed to be a carrier, showed only a slightly skewed pattern that was within the normal range, suggesting that she might be mosaic for a new mutation. Twenty-four microsatellite markers were typed, with an average spacing of 10cM. Haplotype analysis excluded both *TAZ* and *DMD*, a second X-linked cardiomyopathy locus, and identified a 40Mb pericentromeric candidate interval. Sequence analysis of two functional candidate genes within this region was normal. Further families are now sought to help narrow the critical interval for this male-lethal phenotype and pinpoint the underlying molecular defect.

Comprehensive Heritability and Linkage Analysis for ECG Measurements in Extended Mexican American Families. *U. Broeckel¹, J.T. Williams², A. Matter¹, M.P. Stern², J. Blangero², R.T. Bauer², J.W. MacCluer².* 1) Human and Molecular Genetics Center, Medical College of Wisconsin, Milwaukee, WI; 2) Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX.

A number of genes have been identified for monogenic diseases influencing ECG alterations such as long QT syndrome. However, the genetic mechanisms influencing normal variation in ECG measurements have not been described in detail. The aim of this study was to assess genetic influences on ECG measurements as determined by heritability analysis. In addition, we performed a genome-wide linkage analysis in order to identify chromosomal regions influencing these phenotypes.

During a first survey beginning in 1991 resting 12-lead ECGs were obtained in 943 individuals from randomly ascertained families. Individuals with atrial fibrillation and other pathologic changes such as left bundle branch block were excluded from the analysis. Heritability and linkage analyses were performed using the SOLAR software package. A significant heritability was detected for heart rate as determined by averaged R-R interval ($h^2 = 0.39$), PR interval ($h^2 = 0.32$), QRS duration ($h^2 = 0.40$), and QTc ($h^2 = 0.41$). Subsequent linkage analysis identified a number of regions linked to ECG phenotypes. The strongest evidence for linkage was detected for QTc with LOD = 2.93 at chromosome 2 at position 186cM. Additional signals were detected on chromosome 12 (LOD = 1.2), chromosome 17 (LOD = 1.65), and chromosome 22 (LOD = 1.62). Interestingly, the chromosome 17 locus overlaps with a previously described monogenic form of long QT syndrome (KCNJ2; LQT7). In addition, we identified one single linkage signal for QRS duration on chromosome 15 (LOD = 2.94) at position 139cM.

In conclusion, the most frequently determined ECG measurements show a significant heritable component in extended Mexican American families. Suggestive linkage signals were detected for QTc as well as QRS duration. In addition, we will present further data on the 10 year follow-up ECGs in order to describe the potential heritable components with regard to changes over time.

Mapping Of A Novel Gene For Hypertrophic Cardiomyopathy at Chromosome 1q32. *M. Ismail¹, N. Akhtar², A. Abid¹, S. Khaliq¹, S.Q. Mehdi¹.* 1) Biomedical and Genetic Engineering Laboratories, Islamabad, Pakistan; 2) Department of Cardiology, KRL General Hospital, Islamabad, Pakistan.

Hypertrophic cardiomyopathy (HCM) is a clinically and genetically heterogeneous disease with an autosomal dominant mode of inheritance. It is primarily a disorder of the myocardium, characterized by hypertrophy. In the absence of other conditions such as hypertension, aortic valve stenosis or thyroid disease usually the left ventricle is affected. Recent studies suggest that the prevalence of HCM is about 0.2% in the general population. To date, at least 11 disease-causing genes encoding sarcomere proteins, have been identified. Here we report a three-generation Pakistani family with the history of sudden cardiac death inherited in autosomal dominant manner. The family consists of 7 affected and 11 normal individuals. Linkage analysis was carried out to map the disease-causing gene for this family. Genomic DNA from each individual was genotyped for microsatellite markers for all the known HCM loci. A maximum two point lod score of 4.58 was obtained with marker D1S1660 at chromosome 1q 32.2. The disease region was defined by getting proximal and distal cross-overs with markers D1S1189 and D1S1678 respectively that is approximately 13.06 cM. A candidate gene TNNT2 (cardiac troponin T) present in this region was screened for mutation by heteroduplex analysis and direct sequencing. No disease-associated mutation was found in the TNNT-2 gene. Further investigations are in progress to identify the disease-causing gene for this family.

Linkage and association of the *Growth Hormone Secretagogue Receptor (GHSR, Ghrelin receptor)* gene in human obesity. A.E. Kwitek¹, A. Baessler¹, M.J. Hasinoff¹, M. Fischer¹, W. Reinhard², G.E. Sonnenberg¹, M. Olivier¹, J. Erdmann³, H. Schunkert³, A. Doering⁴, H.J. Jacob¹, A.G. Comuzzie⁵, A.H. Kissebah¹. 1) Medical College of Wisconsin, Milwaukee, WI; 2) University of Regensburg, Regensburg, Germany; 3) University of Luebeck, Luebeck, Germany; 4) GSF Institute, Neuherberg, Germany; 5) Southwest Foundation for Biomedical Research, San Antonio, Texas.

The growth hormone secretagogue receptor (GHSR, ghrelin receptor) plays an important role in the regulation of food intake and energy homeostasis. *GHSR* lies on human chromosome 3q26, within a QTL strongly linked to multiple phenotypes related to obesity and the metabolic syndrome. Because its biological function and location make it an excellent candidate gene, we tested the relationship between common single nucleotide polymorphisms (SNPs) in *GHSR* and human obesity. We performed a comprehensive analysis of SNPs, linkage disequilibrium (LD), and haplotype structure across the entire *GHSR* gene region (99.3 kb) in 178 pedigrees with multiple obese members as well as in an independent sample of the general population (WHO MONICA Study). Analyses included variance component analysis and family-based association tests. The LD analysis revealed a disequilibrium block consisting of five SNPs, consistent in both study cohorts. We found linkage between all five SNPs and BMI and, furthermore, provide suggestive evidence for transmission disequilibrium for the minor SNP-alleles and the two most common haplotypes with the obesity affection status in the family cohort. Replication of these findings in the general population resulted in stronger evidence for association of the SNPs and haplotypes with the disease. To our knowledge, these data are the first to demonstrate linkage and association of SNPs and haplotypes within the *GHSR* region and human obesity. The finding of linkage, together with both significant transmission disequilibrium in families and replication of this association in an independent population, provides evidence that common SNPs and haplotypes within the *GHSR* region are involved in the pathogenesis of human obesity.

Genome Scan for Calcified Atherosclerotic Plaque of the Abdominal Aorta: Evidence for Linkage to Chromosomes 7p and 9q in the NHLBI Family Heart Study. *L.A. Lange¹, J.J. Carr¹, I.B. Borecki², G. Heiss³, C.E. Lewis⁴, J.B. Wilk⁵, S.C. Hunt⁶, J.E. Hixson⁷, D.K. Arnett⁸, E.M. Lange¹, J.H. Eckfeldt⁸, L.E. Wagenknecht¹, for the FHS Investigators.* 1) Wake Forest University, Winston-Salem, NC; 2) Washington University, St. Louis, MO; 3) University of North Carolina, Chapel Hill, NC; 4) University of Alabama, Birmingham, AL; 5) Boston University, Boston, MA; 6) University of Utah, Salt Lake City, UT; 7) University of Texas, Houston, TX; 8) University of Minnesota, Minneapolis, MN.

Calcified atherosclerotic plaque in the abdominal aorta has been demonstrated to predict cardiovascular morbidity and mortality and is associated with other measures of atherosclerosis including calcified plaque in the coronary arteries. The NHLBI Family Heart Study (FHS) recruited large extended families from the original FHS cohort and measured atherosclerotic plaque in the infrarenal abdominal aorta and proximal common iliac arteries with multi-detector CT. Quantity of calcified atherosclerotic plaque was measured by Agatston score, modified to account for slice thickness, and then log-transformed (the value 0.50 was first added to observations of zero). We used variance components linkage analysis, adjusting for age and sex, with 579 microsatellite markers (Marshfield set 10.0) to identify chromosomal regions that may harbor genes influencing atherosclerosis in 415 Caucasian pedigrees (2,337 participants with CT data). The mean age (SD) was 57.3 (13.2) years (range 31 - 93); 55% of the cohort was female. The prevalence of calcified atherosclerotic plaque 0 (and median Agatston score in those with plaque) was 74% (1806) in men and 67% (708) in women. The strongest evidence for linkage was observed on chromosomes 7p21.3 (between D7S2201 and GATA119B03; LOD=2.15) and 9q31.1 (near GGAA22E01; LOD=2.10). A potentially important candidate gene for aortic calcification, IL-6, lies under the linkage peak for chromosome 7. This represents the first genome-wide linkage analysis of calcified atherosclerotic plaque of the abdominal aorta. These results suggest that at least two chromosomal regions may harbor genes associated with the quantity of calcified plaque.

A new locus for autosomal dominant mitral valve prolapse on chromosome 13q. *M. Leyne*¹, *F. Nesta*^{1,2}, *C. Simpson*¹, *R. Levine*^{1,2}, *S.A. Slaugenhaupt*^{1,2}. 1) Center for Human Genetic Research, Massachusetts General Hosp, Boston, MA; 2) Harvard Medical School, Boston, MA.

Mitral valve prolapse (MVP) is a very common cardiac disorder in humans, occurring in approximately 2.4% of the general population. The condition is characterized by systolic billowing of the mitral leaflets into the left atrium, often accompanied by mitral regurgitation. To date, two MVP loci have been identified. The first locus, *MMVP1*, was mapped to chromosome 16p11.2 in 1999 and in 2003 our lab identified a second MVP locus, *MMVP2*, on chromosome 11p15.4. During our MVP studies we identified a single large pedigree containing 46 individuals and excluded linkage of this family to both *MMVP1* and *MMVP2*. Thus, we performed a genome scan using the ABI Prism Linkage Mapping Set v.2 and found linkage of MVP to *D13S265* (NPL = 6.62, $p < 0.004$). We then genotyped additional markers in this region and confirmed the localization of *MMVP3* to chromosome 13q31.3-13q32.1 (NPL 18.41, $p < 0.0007$). The *MMVP3* locus is defined as an 8.2Mb region between the markers *D13S794* and *D13S786*. The current transcript map for the candidate region contains 16 genes and shows synteny to mouse chromosome 14. We are currently using novel methods in order to prioritise and initiate mutation screening of the candidate genes. Our results confirm the genetic heterogeneity of MVP and set the stage for the molecular identification of genes that play a role in the pathogenesis of this common cardiac disorder. Determination of the genetic basis of MVP is important because the disease often manifests clinically in the 5th or 6th decade of life through presentation as a severe cardiac event. Additionally, genetic analysis of MVP provides an opportunity to explore clinical findings that are currently of uncertain significance. Our familial studies have revealed individuals with variations of mitral valve morphology that do not meet standard criteria for MVP but resemble the pattern seen in fully affected family members. The ability to identify such precursor valve morphologies in younger, genetically susceptible individuals could further enhance our ability to evaluate patients at risk of developing fully expressed MVP.

Hemoglobin cluster & erythropoietin receptor genes may be related to red blood cell size and count: genome-wide scans in the Framingham Study. *J-P. Lin¹, C.J. O'Donnell², L. Jin³, C. Fox², L.A. Cupples⁴.* 1) DECA/NHLBI, NIH, Bethesda, MD; 2) Framingham Heart Study/DECA/NHLBI/NIH, Framingham, MA; 3) Center for Genome Information, University of Cincinnati, Cincinnati, OH; 4) Department of Biostatistics, Boston University, Boston, MA.

Hematocrit (HCT) levels are associated with different kinds of vascular diseases and all cause mortality. We carried out genome scans on HCT and hemoglobin (HGB), a correlated trait. The results of the two scans were not similar and no evidence suggestive of linkage was found on the chromosome regions where some important candidate genes are located. Other studies indicated red blood cell count (RBC) and size (MCV, mean cell volume and MCH, mean cell HGB), traits related to HCT and HGB, have even stronger genetic components and worth exploring. Most importantly, RBC count and size are major criteria for anemia and related hematology disease diagnoses. So far, no genome scan has been reported to determine the genetic components on those elements. We carried out 10 cM genome-wide scans on RBC, MCV and MCH in a community-based Caucasian cohort, the Framingham Study, using 330 families with 1144 individuals being genotyped and phenotyped. By variance-component linkage methods implemented in SOLAR, the heritabilities were estimated as 56%, 51% and 51% for RBC, MCV and MCH. On the RBC scan, we found maximum lod 3.2 on chromosome 19, 24 cM. Within this area there lies an important candidate gene, erythropoietin (EPO) receptor. On the MCV and MCH scans, both had a maximized lods on chromosome 11, 9 cM with lods 3.8 and 3.6 (empirical p-value method). Right under the peak area, there is hemoglobin cluster - several -like genes which are important candidates for RBC size. We also found other regions with lods > 1.5 on chromosome 12 for RBC, chromosome 1 for MCV and chromosome 18 for MCH. Our results suggest that gene(s) of hemoglobin cluster may have a major effect on the RBC size, whereas, the EPO receptor may be related to RBC count. Further studies would be worthwhile to determine the relationships between RBC size and count and positional candidate genes.

QTL mapping of heart disease modifiers in a mouse model of cardiomyopathy. *F.C. Noonan-Wheeler¹, K.M. Carlson¹, L. Fernandez², H.A. Rockman², D.A. Marchuk¹.* 1) Molecular Genet and Microbiol, Duke University, Durham, NC; 2) Medicine, Duke University, Durham, NC.

The progression from myocardial hypertrophy to heart disease and ultimately to heart failure is a complex process, involving genetic and environmental factors. Elucidating the genetic components contributing to heart disease has been difficult, due largely to the heterogeneity of human populations. Animal models can overcome this difficulty. Transgenic mice with cardiac-specific overexpression of calsequestrin (CSQ), a Ca²⁺ binding protein, develop many of the phenotypes observed in human heart failure including cardiac hypertrophy, dilation and premature death. We found strain specific differences in cardiac function and survival, indicating the presence of genetic modifiers of the heart disease phenotype. We have taken an unbiased genomic approach to the identification of these modifier genes. Our lab is using a reciprocal backcross strategy to map QTLs in the DBA and AKR strains. CSQ+ DBA mice were bred to wild type AKR mice to produce heterozygous F1 progeny. The CSQ+ F1 mice were backcrossed to generate F1/AKR and F1/DBA mice for the linkage scan. We used three quantitative traits, survival and two measures of heart function, fractional shortening and left ventricular-end diastolic diameter to measure linkage. We have previously reported results from the F1/AKR backcross in which 4 QTLs were mapped (LeCorvoisier et al. 2003). Here we report the results of a genome-wide linkage scan in the backcross to DBA. We identified one locus on chromosome 3 that showed linkage to all three traits and one additional locus linked just to heart function. The chromosome 3 QTL appears to be the same locus that we mapped in a different cross using C57/Bl6 and DBA (Suzuki et al. 2002), suggesting that a homozygous DBA genotype at this locus has a protective effect on heart function. Identification of the genes at these QTLs in the mouse will allow us to study the corresponding human genes in our heart disease population. We will perform association studies with polymorphisms in candidate genes to determine which genes and SNPs are functioning as modifiers of the heart failure phenotype.

Evidence for lipid phenotype loci on chromosomes 3q and 7p in the GENECARD study of early onset coronary artery disease. *S.H. Shah¹, D.C. Crossman², J.L. Haines³, C.J.H. Jones⁴, V. Mooser⁵, C.B. Granger¹, J.M. Vance¹, W.E. Kraus¹, E.R. Hauser¹.* 1) Duke University Medical Center, Durham, NC; 2) University of Sheffield, Sheffield, UK; 3) Vanderbilt University, Nashville, TN; 4) University of Wales College of Medicine, Cardiff, UK; 5) GlaxoSmithKline, Inc, RTP, NC.

Background: Coronary artery disease (CAD) and dyslipidemia have strong genetic components. We hypothesize that incorporating lipid phenotypes increases power to map CAD genes, narrows linkage peaks and identifies subsets for study. **Methods:** The aim of GENECARD is to identify genes for early onset CAD using linkage analysis. We performed quantitative trait loci (QTL) mapping and ordered subset analysis (OSA) on 420 GENECARD families (at least 2 siblings with CAD before age 51 in men, 56 in women) using lipids and 390 microsatellite markers. OSA defines more homogeneous subsets and calculates lod scores after ranking families by mean values of a covariate. This results in an overall lod and a maximum subset lod (the highest lod for subsets of families with most extreme covariate means). QTL mapping used SOLAR variance components analysis. **Results:** Two regions with high lod scores on the overall scan showed increased linkage for CAD using OSA: chromosome 3q (max lod 4.2, overall 2.4 $p=0.02$ in families with lowest total cholesterol (TC)) and chromosome 7p (max lod 3.6, overall 1.2 $p=0.03$ in families with highest TC). QTL mapping showed linkage to chromosome 3q (lod 1.4) for high-density lipoprotein (HDL) to TC ratio, and on chromosome 7p (lod 2.0) for TC. Families comprising the peak on 3q have significantly fewer CAD risk factors.

Conclusions: We found evidence of linkage for early onset CAD to chromosome 3q in families with fewer CAD risk factors, and a QTL for HDL/TC ratio in the same region, possibly due to a concentrated non-lipid-related genetic effect on CAD. The linkage to chromosome 7p in families with the highest TC, corroborated by a QTL for TC, may represent a lipid phenotype predisposing to early CAD. This suggests etiologic heterogeneity in our study, possibly due to differential lipid phenotypes. Candidate genes such as apoD and adiponectin reside near these regions and are being studied.

Principal Component Analysis of Quantitative Trait Loci for Adenovirus Gene Therapy. *S.K. Musani¹, H-G. Zhang², H-C. Hsu², N. Yi¹, D.B. Allison¹, J.D. Mountz².* 1) Section on Statistical Genetics, University of Alabama at Birmingham, Birmingham, AL; 2) Department of Medicine, University of Alabama at Birmingham, Birmingham, AL.

Generation of cytotoxic T-cells (CTL) is the most crucial aspect of the immune response to adenovirus (Ad) gene therapy. Other related, albeit minor responses that occur simultaneously include; changes in the sera levels of interferon gamma (IFN-), tumor necrosis factor alpha (TNF-) and interleukin-6 (IL-6). To better understand the genetic network underlying these simultaneous events, data on the -galactosidase (LacZ) expression in the liver, levels of CTLs and sera cytokines from 20 strains of C57BL/6 DBA/2 (BD) recombinant inbred (RI) mice 30 days post intravenous injection of AdLacZ were analyzed. A number of quantitative trait loci (QTLs) affecting each of these variables individually have been reported before, but because these variables are highly correlated, the present study applied principal component analysis to a genome-wide search for QTLs affecting a combination of the original variables. Principal component analysis classified variation in immune response to Ad gene therapy after 30 days into 2 independent principal components: the first principal component (PC1) extracted information on sera cytokines mainly and the second principal component (PC2) extracted information on LacZ expression and CTL production. Maximum likelihood based interval mapping revealed two QTLs on chromosomes 7 and 9 at positions 57.5 cM and 41.01 cM, respectively, to be significantly associated (LOD2.0) with PC1, whereas five QTLs were significantly associated with PC2, which mapped on chromosomes 12 (23.01 and 31.01 cM) and 15 (29.21, 36.01 and 56.31 cM). These results demonstrate the utility and potential importance of principal component analysis in mapping QTL affecting complex multiple correlated traits.

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Pseudo: A program for fast calculation of empirical p-values for genomewide non-parametric tests of linkage.

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The calculation of genomewide empirical p-values for non-parametric linkage tests continues to present significant computational challenges for many complex disease mapping studies. Once a genome scan is performed, it is common practice to determine significance of lod score peaks using simulation, specifically by repeated linkage analysis of thousands or even millions of datasets generated by gene-dropping to reproduce the underlying null distribution. This empirical distribution can be used to estimate how frequently peaks of similar or greater magnitude occur by chance in data of similar structure. One limitation of this approach is the fact that individual simulations may take hours or days of computational time to complete, making it impractical to perform enough simulations to adequately evaluate significant findings. In these simulation studies, most computational effort is expended in subroutines that compute family-specific z-scores. An efficient method for sampling null genome scans is to pre-calculate family-specific z-scores for a modest number of replicates for each family and then to randomly sample from this pool in order to generate "pseudosimulations". These pseudosimulations can then be used to assign an empirical p-value to the lod score of interest. We have implemented this idea in a new program called Pseudo. Pseudo is able to compute empirical p-values extremely efficiently relative to conventional methods that utilize gene-dropping. While actual time savings will depend on the underlying data set, we have been able to generate 1000 pseudosimulations in an hour using <50M of memory for a data set that previously required 24 hours and 1.5G of memory per simulation, thus realizing a 24,000-fold speed gain while using 30 times less memory. In addition to extremely efficient calculation of empirical p-values for individual affection variables, Pseudo is able to assess significance of multiple correlated affections, produces a graphical summary of results, and provides an estimated variance for empirical p-values. Pseudo is freely available for download on our web site at <http://www.sph.umich.edu/csg/abecasis/Pseudo>.

A genome-wide scan of serum cholesterol level in young-onset hypertension families. *C. Chung¹, C.S.J. Fann¹, C.L. Hsu¹, J.W. Chen², Y.S. Jong³, H.M. Lo³, F.M. Ho³, C.K. Kang⁴, C.C. Chen⁵, H.C. Change⁵, W.H. Pan¹.* 1) Institute of Biomedical Sciences, Academia sinica, Taipei, Taiwan; 2) Cardiovascular Research Center, National Yang-Ming University, Taipei, Taiwan; 3) Tao-Yuan General Hospital Department of Health, Taiwan; 4) Min-Sheng Hospital, Taoyuan, Taiwan; 5) Lin-SHin Hospital, Chung-Li, Taiwan.

Elevated serum levels of low density lipoprotein cholesterol (LDL-C) and cholesterol are important risk factors for cardiovascular disease (CVD), which are well-established intermediate traits that may be influenced more directly by lipid metabolizing genes than clinical CVD end points. To identify the quantitative trait loci influencing total serum cholesterol and LDL-C levels, we conducted genome-wide linkage analyses in 1337 individuals from 245 families of the young-onset hypertension study which was carried out in three Hakka communities located in the northwestern Taiwan. The marker set of deCODE genetics was typed for the study subjects, which defines a roughly 10 cM resolution human index map. Evidence of linkage was assessed with a variance component method using SOLAR software. There was a strong evidence of linkage found on chromosome 6q27 for LDL-C with a LOD score of 3.42 adjusted for sex and body mass index. Other chromosomal regions with highly suggestive evidence of linkages for LDL-C and cholesterol included 8q11 (LOD 2.00), 17q25 (LOD 2.46), and 4p16 (LOD 1.86). Three candidate genes of interest are found in these regions: Apolipoprotein(a) (Lp(a)), Plasminogen (PLG) near 6q27 and Cholesterol 7- α -hydroxylase (CYP7A1) near 8q11. To establish the relevance of Lp(a), PLG, and CYP7A1 for cholesterol, we performed association study using Quantitative (Trait) Transmission/Disequilibrium Test (QTDT) and Family Based Association Test (FBAT) software after genotyping 25 single-nucleotide polymorphisms (SNPs) around three candidate regions. There are two SNPs showed significant association at PLG ($Z=2.47$, $p=0.013501$) and CYP7A1 ($Z=-2.943$, $p=0.003247$), respectively. These results have thereby allowed us to significantly reduce the number of potential candidate regions and to prioritize genes for further mutation screening.

Evidence for a QTL on 12q23 influencing triglyceride and high density lipoprotein cholesterol levels in the

HERITAGE Family Study. *M. Feitosa¹, T. Rice¹, K.E. North², T. Rankinen³, A.S. Leon⁴, J.S. Skinner⁵, J.H.*

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To determine whether a QTL influenced the variation of triglyceride (TG) and high density lipoprotein cholesterol (HDLc) levels, bivariate linkage analysis was employed using 654 makers. Covariate effects of age, sex, hormonal use, smoking, fasting insulin levels and body mass index (BMI) were removed and the residuals were used for SOLAR univariate and bivariate variance component linkage analysis. A maximum genome wide bivariate LOD score of 3.0 was found on chromosome 12q23-q24 (at 108cM) for TG and HDLc in White families (from 99 nuclear families with 606 parent-offspring and 383 sibling pairs). This linkage peak is substantially higher than the univariate linkage at the same chromosome location for each trait (TG: LOD = 1.98, HDLc: LOD = 2.04). The genetic correlations between baseline TG and HDLc were -0.14, for the residual component (r) and -0.33 for the QTL (Q_{TL}). The complete pleiotropy ($Q_{TL} = 1$) and the coincident linkage QTL ($Q_{TL} = 0$) hypotheses were both rejected ($p < 0.001$). In conclusion, these results suggest incomplete pleiotropy for a QTL on 12q23-q24 influencing the plasma levels of TG and HDLc. A putative nearby positional candidate gene is the class B scavenger receptor (SR-BI), which has a high affinity binding for HDLc and mediates selective cholesterol uptake.

A QTL for left ventricular mass on chromosome 12p in American Indians. *H.H.H. Göring¹, V.P. Diego¹, S.A. Cole¹, L. Almasy¹, T.D. Dyer¹, K.E. North², T.K. Welty³, L.G. Best³, R.R. Fabsitz⁴, B.V. Howard⁵, E.T. Lee⁶, S. Laston¹, B. Dyke¹, J.W. MacCluer¹, R.B. Devereux⁷.* 1) Dept. of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 2) Dept. of Epidemiology, University of North Carolina, Chapel Hill, NC; 3) Strong Heart Study Dakota Center, Rapid City, SD; 4) National Heart, Lung, and Blood Institute, Bethesda, MD; 5) MedStar Research Institute, Hyattsville, MD; 6) University of Oklahoma Health Sciences Center, Oklahoma City, OK; 7) Cornell University Medical Center, New York, NY.

Left ventricular mass (LVM) is a clinically important phenotype for assessing the risk of cardiovascular morbidity and mortality. Identification of the genetic factors that influence an individual's LVM may therefore provide crucial knowledge about heart disease and treatment. We have measured LVM (adjusted for height) in participants in the Strong Heart Family Study, a multi-center study of cardiovascular disease (CVD) risk factors in large, randomly ascertained pedigrees of American Indians from Arizona (AZ), North/South Dakota (DK), and Oklahoma (OK). Each individual's phenotype was adjusted for sex and age and normalized prior to analysis. Data from the 3 centers were analyzed separately as well as jointly. 1915 phenotyped individuals from 69 pedigrees in total were included in the analyses, with nearly equal sample sizes from the 3 centers. The heritability of LVM was estimated as 0.38, 0.46, 0.45 and 0.43 in the AZ, DK, OK and combined sample, respectively. Genome-wide variance components linkage analysis yielded a lod score of 3.1 on chromosome 12p (between markers D12S364 and D12S310) in the AZ sample. This finding was replicated with a lod score of 3.1 at the same position in the DK sample. In the OK sample, the lod score at the same position was 0.3. The 3 datasets were subsequently combined. Analysis of the combined sample yielded a lod score of 5.4. No other significant lod scores were obtained in the individual datasets or the combined sample. In summary, our results provide overwhelming evidence for a major locus for LVM, a strong predictor of CVD, on chromosome 12p in American Indians.

Evidence for Ethnic Specific QTLs for CRP levels: The IRAS Family Study. *J.I. Rotter^{1,2}, S. Beck³, X. Guo^{1,2}, C.D. Langefeld³, K.D. Taylor^{1,2}, F-C. Hsu³, L. Wagenknecht³, S. Rich³, M.F. Saad², Y.D. Chen^{1,2}.* 1) Medical Genetics, Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) UCLA, Los Angeles, CA; 3) Wake Forest University School of Medicine, Winston-Salem, NC.

Increased C-reactive protein (CRP) levels have been reported to predict coronary heart disease, stroke, peripheral vascular disease, and diabetes, and are associated with features of the metabolic syndrome. Family-based studies have shown that plasma levels of CRP are influenced strongly by genetic factors, including variation in the CRP gene itself. As part of the IRAS Family Study, the NHLBI Mammalian Genotyping Service (MGS) performed a 10 cM genome scan on 1520 individuals (Hispanic American (HA): 1135; African American (AA): 567) from 130 families (HA: 88; AA: 42). A variance components approach (SOLAR) was used for analysis on plasma CRP (g/ml) levels (determined by ELISA), with age, sex, and BMI included as covariates to adjust for possible confounding effects. These analyses revealed a potential quantitative trait locus (QTL) on chromosomes 1q21-25 near D1S1589 (LOD=2.66 at 187 cM) and 11p15 near D11S1984/D11S2362 (LOD=2.77 at 6 cM) that contributes to variation in CRP level in the AA sample. Only very modest evidence of linkage to CRP levels in these regions were observed in the HA families (1q21-25 LOD=0.25 and 11p15 LOD=0.13). Interestingly, several studies have reported linkage of type 2 diabetes to the 1q21-25 region and the CRP gene is within 1q23. The strongest support for linkage in HA was on chromosome 4q23-25 (LOD=2.11, 109 cM). This region is rich in immunological candidate genes such as the FCgamma family. When combining the HA and AA data and further including ethnicity as a covariate, the LOD score remained 2.06 on chromosome 1q in the entire sample. In conclusion, there is evidence supporting the existence of QTLs on chromosome 1 and on chromosome 11 that contribute to the variation of CRP levels in AA while HA show linkage to chromosome 4, providing evidence for genetic heterogeneity between the two ethnic groups.

Genetic analysis of HNF4 polymorphisms in Caucasian American type 2 diabetes. *A.M. Bagwell^{1, 4}, J.L. Bento^{2, 4}, J.C. Mychaleckyj^{3, 4}, B.I. Freedman³, D.W. Bowden^{1, 2, 3, 4}.* 1) Molecular Genetics Program; 2) Department of Biochemistry; 3) Department of Internal Medicine; 4) Center for Human Genomics, Wake Forest University School of Medicine, Winston-Salem, NC.

Hepatocyte nuclear factor 4 alpha (HNF4), the gene for maturity-onset diabetes of the young type 1 (MODY1) subtype of type 2 diabetes mellitus (T2DM), lies within the T2DM-linked region on chromosome 20q12-13 and is therefore a positional candidate for later-onset T2DM. Only a few rare coding mutations have been identified in HNF4, but recent studies suggest that single nucleotide polymorphisms (SNPs) located near the HNF4 alternate promoter P2 are associated with diabetes susceptibility in Finnish and Ashkenazi populations. We genotyped 23 SNPs covering 111 kb which includes the HNF4 gene and tested them for association with T2DM in a collection of 300 Caucasian T2DM patients with end-stage renal disease (ESRD) and 310 controls. In contrast to previous reports of association, our single SNP analysis provided no evidence for association with T2DM, with P-values ranging from 0.06-0.99. However, haplotype analysis of SNPs in two separate linkage disequilibrium blocks, one encompassing the P2 promoter and the second in the exon 4 region of HNF4, identified statistically significant differences in haplotype frequencies between cases and controls ($P < 0.0001$). The haplotype analysis identified two uncommon risk haplotypes (2.36% and 2.24% of chromosomes) in the P2 block and two uncommon protective haplotypes (7.14% and 5.02% of chromosomes) in the exon 4 block that account for the observed haplotypic associations. DNA sequencing did not identify SNPs in P2 or mutations in exon 4. The observed associations with haplotypes in the absence of single SNP associations suggest that a combination of noncoding SNPs may drive the observed associations with the phenotypes. In addition, evidence that other genes in 20q12-13 are associated with T2DM in our subjects suggests that the diabetes phenotype may be due to the contribution of multiple genes. Together, these results suggest that T2DM linked to 20q12-13 is a heterogeneous disease in which different populations may have different T2DM susceptibility loci.

Parametric linkage analysis of type 2 diabetes (T2DM) replicates 2 loci and reveals a pattern of heterogeneity in pedigree-specific analysis. *W. Blaum*^{1,2}, *K. Hunt*², *M.P. Stern*². 1) Institut fuer Humangenetik, Charite Berlin, Germany; 2) Dev of Epidemiology, Univ of Texas Health Science Center San Antonio.

Of the 39 Mexican-American extended pedigrees from the San Antonio Family Diabetes Study we selected for parametric linkage analysis 7 pedigrees in whom an autosomal dominant mode of inheritance for T2DM was suggested. WHO and ADA criteria were used to define T2DM. The 7 pedigrees contained 242 persons of whom 99 were affected by WHO criteria (68 by ADA). 182 subjects were genotyped by CIDR with a set of 382 microsatellites. Four runs of two-point (tpt) analysis (WHO and ADA criteria with and without age-adjustment) were performed using LINKAGE. Regions for which at least two runs showed LODs > 1 and no run contradicted linkage are reported and were selected for parametric multi point (mp) analysis using GENHUNTER2. Markers that met these criteria included D9S1779 (max. tpt LOD: 1.35), D9S1871 (1.13) and D9S2169 (1.43) on chr9p as well as D20S478 (1.09) on chr20. Mp analysis showed a LOD of 1.45 near D9S2169 and of 1.3 near D20S478 in all 7 families. The 2p HLODs (GENHUNTER2) indicate heterogeneity in both regions: 2.1 (=0.53) on chr9p and 1.51 (=0.27) on chr20. We thus examined markers in individual families where at least one tpt run showed suggestive linkage (LOD > 1.9), at least one a LOD > 1, and no run showed a LOD < 0.588 (corresponding to a p-value of 0.05). Of 5 results that met these criteria the most interesting were a tpt LOD of 2.94 (family #2) at marker D9S2169 and of 2.69 at D20S478 (family #9). All flanking markers in these families also showed LODs > 1. In mp analysis family #2 shows a LOD of 2.93 near D9S2169 and family #9 one of 2.44 near D20S478. Results on chr9p replicate previous findings in Mexican-Americans and American-Caucasians while the region on chr20 has recently been linked to T2DM in Finns and Ashkenazim Jews. An interesting positional candidate in the latter region is HNF4 (MODY 1). Our study thus proved useful in the identification of both, susceptibility loci for T2DM and small(er) sets of individuals for whom a further investigation seems promising.

Extended haplotype in the PKLR region on 1q21 is associated with type 2 diabetes. *R.L. Craig¹, Z. Zhang¹, W.S. Chu¹, H. Wang¹, S.C. Elbein²*. 1) Internal Medicine, UAMS, Little Rock, AR; 2) Central Arkansas Veterans Healthcare System, Little Rock, AR.

Type 2 diabetes has been mapped to chromosome 1q21-q24 by multiple laboratories. Pyruvate kinase, a key glycolytic enzyme expressed in liver and pancreatic beta cells, is encoded by PKLR and is located within the linked region. We previously reported an association of 4 SNPs within the PKLR gene with T2DM. To identify the extent of that association, we selected 65 SNPs over a 400 kb region surrounding PKLR from the public database. Only 17 SNPs were polymorphic and could be successfully typed using Pyrosequencing. We initially typed pools of 192 unrelated, non-diabetic Caucasian control individuals and 192 Caucasian individuals with T2DM, and confirmed differences of over 5% in individual samples. We identified 9 additional SNPs that were associated with T2DM ($p < 0.05$) in a population that was expanded from our original report. The associated SNPs extended from 8 kb telomeric to 50 kb centromeric to PKLR, and encompassed at least 4 additional genes. Within this region, all associated SNPs showed strong linkage disequilibrium ($r^2 > 0.75$) and most associated SNPs were in complete linkage disequilibrium. The strongest associations showed p values of 0.007 (OR=1.6) and tagged the extended haplotype. In contrast, only 1 SNP in this region showed neither association nor linkage disequilibrium by r^2 . No association was found when the most strongly associated PKLR SNPs were tested in 260 African American diabetics and 160 African American controls, however. We have confirmed our earlier findings of an association using additional markers and an expanded population. However, extensive disequilibrium will make identification of the causative variant in this region challenging. The lack of association in African American subjects may suggest that the responsible SNP is 5' or 3' to PKLR. Work is in progress to resequence each gene in this region in Caucasian individuals with each haplotype to identify additional variation given the paucity of validated and 2 hit SNPs in the current database.

Examination of candidate chromosomal regions for type 2 diabetes mellitus (T2DM) in Kuwaiti population. *M.M. El-Zawahri¹, M.S. Al-Dabbous¹, M.A. Taha², M. Al-Aroug³*. 1) Dept Biological Sci, Fac Sci, Kuwait Univ, Safat, Kuwait; 2) Dept Statistics & Operation Research, Fac Sci, Kuwait Univ, Kuwait; 3) Alameery General Hospital, Ministry of Health, Kuwait.

Type 2 diabetes mellitus (T2DM) shows multifactorial etiology with strong evidence for many genes interact not only with each other but also with environmental factors. It accounts for approximately 90% of diabetes cases. Kuwaiti population is a small and multiplex families from three main Asian ancestral populations (Saudi Arabia, Iran and Iraq). The rate of consanguineous marriages has reached 54.5% within the Kuwaiti families. The prevalence of T2DM in Kuwaitis is two fold higher than worldwide prevalence average. We genotyped markers in nine chromosomal regions (1q, 2p, 2q, 3q, 5q, 7q, 8p, 9q, and 20q), previously reported as supporting linkage with T2DM, in a panel of 42 large Kuwaiti pedigrees in which diabetes segregates consistent with an autosomal dominant (AD) pattern. Candidate regions on 3q, 5q, 7q and 9q yielded little evidence for linkage. Regions on 1q (MLS=0.96), 2p (MLS=1.11) and 2q (MLS=0.84) gave only suggestive evidence of linkage. While as regions on 8p (MLS=3.71) and 20q (MLS=3.35) gave significant evidence of linkage. These results provide strong evidence to support 8p and 20q as candidate chromosomal regions for susceptibility loci for type 2 diabetes mellitus in Kuwaiti population. In conclusion, linkage evidence provided in this study is particularly important given the evolutionary history of this multiplex Kuwaiti population and its well documented historic contribution ; Kuwaitis have one of the most highest rates of diabetes in the world. This work was supported by College of Graduate Studies, Kuwait University, Kuwait.

Variation in conserved nongenic sequence upstream of dual specificity phosphatase (DUSP12) gene on chromosome 1q21 is associated with type 2 diabetes. *S.C. Elbein^{1,2}, S.K. Das¹, W.S. Chu¹, Z. Zhang¹, S.J. Hasstedt³.*
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Genome scans in multiple populations spanning 4 ethnic groups have linked chromosome 1q21 to type 2 diabetes (T2DM). We tested 568 SNPs across the 15 Mb linked region and identified a significantly associated ($p < 0.00001$) SNP in pooled DNA. We tested 45 polymorphic SNPs at a mean 4 kb interval over 1 Mb around this SNP and narrowed the associated region to a 50 kb interval encompassing the DUSP12 gene. DUSP12 is expressed in liver and pancreas and was previously shown to regulate glucokinase activity in liver. We screened for mutations in DUSP12 exons, introns, and 2 kb of 5' and 3' flanking regions in 16 cases and 8 controls. We also identified a highly conserved nongenic sequence (CNG) at -8 kb from the ATG start and near the original association. We tested the association of 29 SNPs over 20 kb in 192 Caucasian cases and 192 Caucasian controls, and identified 10 SNPs over 18 kb that were associated with $p < 0.05$. Additional SNPs showed a trend to association. The strongest associations were with a microsatellite within the CNG (-8379) and a SNP at 7581 in the 3' flanking region. The SNPs fell into 5 haplotype blocks defined by D' (confidence intervals). The associated SNPs fell into 2 blocks by D' or 4 bins defined by r^2 . The most associated haplotypes were driven by the CNG microsatellite (-8379) and the 3' (7581) SNP with $OR = 1.7$. Haplotypes of 4-5 markers spanning 3 - 4 bins and 2 blocks showed strong associations with T2DM ($p = 0.0009$ and $p = 0.00015$), with both protective and risk haplotypes. Both the microsatellite and 7581 SNPs were also associated with T2DM in 286 cases and 167 controls of African American ethnicity ($p < 0.02$ for both), but other SNPs were not associated. Furthermore, allele frequencies and associated microsatellite alleles differed from Caucasians. We propose that the 3' (7581) SNP and the CNG variants alter DUSP12 expression and lead to T2DM through altered glucokinase activity in liver and pancreas.

Contribution of CYP19 polymorphisms to the risk of type 2 diabetes stratified by physical activity. C.E.

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Type 2 diabetes mellitus (T2DM) may result from an interaction between genetic susceptibility and environment. A polymorphism in the cytochrome p450 aromatase (CYP19) gene (15q21.1) was found to be linked with T2DM, after conditioning on Calpain-10 (Cox, 1999). In addition, studies have found physical inactivity to be a determinant in T2DM. Despite the importance of both environmental and genetic factors, few studies have examined these factors together. We used the family based association test (FBAT) to look for association of 2 single nucleotide polymorphisms (SNPs) in the CYP19 gene and T2DM in 743 affected Caucasian individuals from 252 families from Colorado. The FBAT allows for missing parental genotypes, and accounts for the relatedness of affected individuals in the analysis. A physical activity history was collected by questionnaire and average METs per week from occupational and leisure activity prior to diabetes diagnosis was calculated. We conducted an analysis overall, and in the upper and lower tertiles of physical activity. In the overall analysis, the Hgal site of CYP19 was associated with T2DM ($p=0.002$) and the rs700518 site showed suggestive evidence for association ($p=0.08$). In the affecteds with high levels of physical activity, the Hgal site was less significantly associated with T2DM ($p=0.03$) and the rs700518 site showed no association ($p=0.46$), results that could be influenced by the reduced sample sizes in each tertile. However, in the affecteds with low levels of physical activity (exposed to the risk of inactivity) the rs700518 site showed significant association with T2DM ($p=0.01$) despite that reduction in sample size. The Hgal site could not be analyzed in the affecteds with lower physical activity due to a small sample size since the 1 (Cys) allele has a frequency of 6%. In conclusion, the CYP19 gene may be associated with T2DM, particularly in individuals with lower levels of physical activity, suggesting a potential gene-environment interaction.

Strong association of intragenic SNPs with type 2 diabetes in the 152 megabase region of chromosome 1q21 in the Old Order Amish. *M. Fu*^{1,2}, *M. Sabra*¹, *C. Damcott*¹, *T.I. Pollin*¹, *S. Ott*¹, *K. Tanner*¹, *J. Wang*¹, *X. Shi*¹, *J. Connell*¹, *B.D. Mitchell*¹, *A.R. Shuldiner*^{1,3}. 1) Dept Med, Div Endocrinology, Diabetes, and Nutrition, Univ Maryland, Baltimore, MD; 2) Division of Endocrinology, the Second Affiliated Hospital, Sun Yat-sen University, Guangzhou, P. R. China; 3) Geriatric Research and Education Clinical Center (GRECC), Veterans Administration Medical Center, Baltimore, MD.

Linkage of type 2 diabetes (T2DM) to chromosome 1q21-q24 has been observed in the Old Order Amish, and other 5 populations. Amish genome-wide and fine-mapping linkage studies revealed evidence for linkage of T2DM and impaired glucose tolerance (IGT) to 1q21 (near D1S2858, LOD = 2.35, P = 0.0008). To positionally clone the T2DM susceptibility gene in the region, we first performed linkage disequilibrium (LD) mapping with SNPs distributed over the broad region of 40 Mb underlying the linkage peak. Additional iterative genotyping of SNPs around positive signals identified three distinct regions associated with T2DM and/or IGT, at 152-153 Mb, 156-161 Mb, and 166-168 Mb. In total, 100 SNPs at inter-marker intervals averaging 40 Kb were genotyped in the 150-153 Mb region in 651 Amish subjects with T2DM (n = 145), IGT (n = 148), and normal glucose tolerance (n = 358). We identified 6 SNPs associated with T2DM, IGT, and T2DM/IGT (P = 0.0006-0.0444) and 4 SNPs associated with IGT and IGT/T2DM (P = 0.0015-0.023). These SNPs were genotyped in an expanded set of 1200 Amish subjects and were found to be significantly associated with glucose area under curve during an oral glucose tolerance test (p = 0.001-0.049), providing additional support for association of the SNPs with glucose homeostasis. The nine significantly associated SNPs were in strong LD (D' 0.9) and formed a haplotype block of approximately 67 Kb. The SNPs were distributed in introns, untranslated regions and within 2 Kb of 7 genes. Association of SNPs to T2DM within the region of high LD has also been shown in Utah Caucasians. In summary, it is likely that more than one gene in the chromosome 1q21-q24 region contributes to T2DM and may reside within a 67 kb region defined by these ongoing LD mapping studies.

Selected Linkage Studies for T2DM to Chromosomes 20, 12 and 17 in Italians. C. Gragnoli^{1,2,3}, E. Milord⁴, J.F. Habener^{1,2,5}. 1) Mol Endocrinology, Mass Gen Hosp, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) Bios Health Center, Rome, Italy; 4) Millennium Ph, Inc, Cambridge, MA; 5) HHMI, Boston, MA.

Type 2 diabetes mellitus (T2D) is a common complex trait disorder. Multiple genome scans have identified different loci in linkage with T2D. Loci on chromosomes 12q15 and 20q have been identified in independent studies. A meta-analysis of four genome scans has reported a linkage on chromosome 17p11.2-q22. Not far away from this region lies the glucagon receptor gene (GCR), on chromosome 17q25. The possibility that the linkage on chromosome 17 might include the GCR gene may not be entirely eliminated, given substantial potential variation in loci localization estimates. In a combined French-Sardinian study of GCR gene there is association of Gly40Ser mutation with T2D, confirmed by an UK study but not by other studies. Our goal was to study these selected regions of chromosome 17, 12 and 20 in a group of Italian patients with late/early-onset T2D by microsatellites genotyping (D17S801, D17S937, D17S180; D12S355, D12S1056, D12S1655; D20S887, D20S109, D20S196) and non-parametric multipoint linkage analysis (Merlin 2000-2002/decode map or Marshfield map) with allele frequencies calculated from sib-pairs data. We have recruited from Rome 174 sib-pairs with T2D, 29 sibpairs/families with early-onset T2D and MODY. The linkage analysis at chromosome 17q25 reported a lod score=-0.22 (P=0.8) in the total T2D sib-pairs, a lod score=-0.09 (P=0.7) in the late-onset T2D group and a lod score=-0.31 (P=0.9) in the early-onset T2D group. At chromosome 12q13.1-13.2 in the total T2D sib-pairs identified a lod score=0.03 (P=0.4), a lod score=-0.02 (P=0.6) in the late-onset T2D group and a lod score=0.67 (P=0.04) in the early-onset T2D group. At chromosome 20q13.1 in the total T2D sib-pairs reported a lod score=0.35 (P=0.1), a lod score=0.32 (P=0.11) in the late-onset T2D group and a lod score=0.11 (P=0.2) in the early-onset T2D group. We can exclude in our patient cohort linkage to T2D in the region containing the GCR gene and on chromosome 20q13.1. Linkage analysis in our patient group suggests that chromosome 12q15 might carry a gene causing T2D in the early-onset Italian T2D cohort.

Further evidence of a Quantitative Trait Locus for Body Mass Index (BMI) mapping to Chromosome 10p and subsequent analysis of the positional candidate gene *GAD2* in a large European Type 2 diabetes sibship resource.

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Type 2 diabetes (T2D) and obesity are closely related phenotypes. In this study we reanalysed genome scan data from 418 microsatellite markers (mean spacing 9.3cM (H)) genotyped in 573 UK sibships multiplex for T2D, using BMI as the outcome quantitative trait. BMI information, available from 1223 T2D subjects (mean (SD) BMI: males 27.8 (4.3) kgm⁻²; females 29.8 (5.6) kgm⁻²), was log-transformed, adjusted for age and sex, and standardised against a representative sample of 14000 UK subjects to account for selected ascertainment. Analysis was performed using MERLIN-REGRESS to implement a modified Haseman-Elston (HECOM) analysis. The only region demonstrating significant linkage was located at 10p11.2-10q11.3. The maximum LOD score (3.74 @ 42 Mb (NCBI34), pointwise asymptotic $p=1.7 \times 10^{-5}$ between D10S208 - D10S196) reached genome-wide significance (empirical $p=0.0158$), with no other region generating LOD scores 1.18. This same region has shown evidence for linkage to obesity/BMI in seven studies to date. In French families showing linkage to 10p, positive associations were reported with variants in the *GAD2* gene. *GAD2* encodes glutamic acid decarboxylase, an enzyme that has been implicated in the gabanergic control of appetite. However, in the UK families we found no evidence for association between any of these variants (-243 AG: $p=0.8$; +61450 CA: $p=0.5$; +83897 TA: $p=0.5$), or their haplotypes and obesity. In addition, using family-based association analysis (QTDT) we found no evidence that these variants accounted for the 10p linkage signal. These data provide strong, replicative evidence for the presence of an obesity gene on chromosome 10p: however, variation at the *GAD2* locus is not likely to be responsible, and linkage must be due to variation in other genes.

Variants at the CTLA4 gene region are significantly associated with both type 1 diabetes and age at diagnosis in Newfoundland. *M. Liu¹, K.S. Wang¹, M.Y. Lu¹, B. Bharaj¹, H.T. Chen¹, J. Pasternak¹, J.A. Curtis², L.A. Newhook², A.D. Paterson^{1, 3}.* 1) Program in Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Department of Pediatrics, Memorial University of Newfoundland, St. Johns, Newfoundland, Canada; 3) Department of Public Health Sciences, University of Toronto, Toronto, Ontario, Canada.

Background: Studies have shown that both type 1 diabetes (T1D) and the age at diagnosis of T1D have a genetic component. The CTLA4 gene is an important negative regulator of the T lymphocyte immune response, and is one of the three confirmed candidate loci (IDDM12) for T1D. However, the relation between the CTLA4 gene and the age at diagnosis of T1D is largely unknown. Method: We genotyped eight SNPs and one (AT)_n microsatellite marker which covered about 45 kb around CTLA4 (2q33). Family-based association analysis was performed with FBAT using 538 extended families (including 628 affected individuals) from Newfoundland, where there is a high incidence of T1D. Age at diagnosis was analyzed as a quantitative trait with an upper limit at 18 years old in order to reduce the effect of extreme values. Since all the markers were in strong linkage disequilibrium (LD) with mean D equal to 0.93, haplotype analysis of all possible groups of markers was also performed. Result: For T1D, four markers (3 SNPs and one microsatellite marker) showed significant association ($p < 0.01$) with the lowest p value equal to 0.0016 for allele 120 of the (AT)_n microsatellite marker (allele frequency 0.029). The most significantly associated haplotype ($p = 0.00021$) was a SNP pair rs231728-rs231806 (frequency 0.036) with excess transmission to affected offspring. For the age at diagnosis of T1D, two SNPs rs231806 (allele frequency 0.59) and rs5742909 (allele frequency 0.089) were significant associated with p values 0.0026 and 0.0037, respectively. Haplotype rs5742909-rs3087243 (frequency 0.078) was most significantly associated with age at diagnosis ($p = 0.0013$). Conclusion: Our data showed that the polymorphisms at the CTLA4 gene region were significantly associated with both T1D susceptibility and the age at diagnosis of T1D in Newfoundland population.

Genetic Analysis of Quantitative Lipid Traits in the Genetics of NIDDM (GENNID) Study. *A. Malhotra, J.K. Wolford.* Translational Genomics Research Institute, Phoenix, AZ.

Lipid levels serve as important markers in a number of diseases with high prevalence, including cardiovascular disease, obesity, and diabetes. Although environmental factors such as diet affect lipid levels, familial aggregation and twin studies have suggested the role of genetic factors and heritability estimates have been found to range from 0.3-0.75. The goal of this study was to identify potential genetic determinants of lipid levels in African Americans, Caucasians, and Hispanics from the GENNID repository, a publicly available resource that contains information on families ascertained with at least two diabetic children. There are a total of 350 families corresponding to approximately 1,750 individuals in our study sample. Regression analyses were performed on low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TG), total cholesterol, LDL/HDL, and TG/HDL and the data were adjusted for covariates, such as age and gender, significantly related to these values. In a segregation analysis of these traits, two-locus models, in some cases, had higher likelihood estimates than the single gene models, but a mode of inheritance could not be inferred. We performed variance components linkage analysis as implemented in the program Genehunter. In African-Americans, the highest multipoint LOD scores were found for LDL/HDL (LOD=1.9) on chromosome 5p, and cholesterol (LOD=1.73) and LDL (LOD=1.54), both on chromosome 19p. Two-point LOD scores of 0.97 for LDL/HDL, 1.32 for cholesterol, and 1.64 for LDL were observed in these locations. The linked region on chromosome 19, which is approximately 10cM from the LDL receptor and the APOE genes, overlaps with results obtained in at least five independent studies, providing further support that 19p harbors a susceptibility locus. No LOD scores greater than 1.5 for HDL, TG, or TG/HDL were found. We are currently performing variance components linkage analysis using Caucasian and Hispanic genotype data.

Genetic analysis of Type 1 Diabetes in an Isolate Population from South America. *N. Pineda-Trujillo*^{1,5}, *F. Uribe*², *V. Balthazar*³, *F. Montoya*⁴, *J.M. Alfaro*³, *G. Latorre*², *A. Villegas*², *J. Ceron*^{1,2}, *A.F. Perez*^{1,2}, *M. Ospina*¹, *A. Naranjo*¹, *A. Serrano*², *D. Castrillon*^{2,3}, *I. Duque*², *A. Abad*², *C.E. Duque*¹, *G. Bedoya*¹, *A. Ruiz-Linares*^{1,5}. 1) Grupo de Genetica Molecular; 2) Medicina Interna-Endocrinologia; 3) Endocrinologia- Pediatria y Puericultura, from Universidad de Antioquia, Medellin, Colombia; 4) Universidad Pontificia Bolivariana, Facultad de Medicina, Medellin, Colombia; 5) Biology (Wolfson House)University College London, London-United Kingdom.

The most important genetic risk for type 1 diabetes (T1D) is conferred by HLA (IDDM1) and it is believed that this locus interact with others to give the T1D phenotype. We have done two separate studies in samples from Antioquia, a population isolate in North West Colombia. The first study involved a set of three families, where one of them presented with 9 out of 16 affected individuals. This family showed a dominant-like inheritance mode with high penetrance (98.5%) and affected individuals were anti-GAD positive. In addition to evaluate previously reported candidate loci on chromosomes 2 and 11, we also evaluated the rest of these two chromosomes with markers from the Linkage mapping set v.2 (Applied Biosystems). We also evaluated STRs on class II HLA. For chromosomes 2 and 11 negative lod scores (Z) were obtained and for HLA low values were obtained. However, a $Z = 2.8$ was found at theta zero in 2pter for marker D2S319. Haplotypes in HLA were randomly transmitted. In the second study we collected one hundred trios (father-mother-affected child) and evaluated D2S319, IDDM2 and IDDM8 loci. All affected children were tested for anti bodies anti-GAD and only 40% were positive. The most significant Pvalue in the TDT was for D2S319 and for IDDM8 with a value of 0.025. Hph I at IDDM2 with a Pvalue of 0.03. After stratifying according to the antibodies result, Pvalues of 0.03, 0.04 and 0.04 for IDDM8, D2S319 and Hph I respectively were obtained. These results suggest that in this Colombian sample there are important effects of at least these three loci affecting the susceptibility for T1D. Including therefore that the new locus (D2S319) truly exists in 2pter.

Stronger evidence for linkage in type 2 diabetes families with mean age-of-diagnosis 55 years of age. *L.J. Scott¹, W.L. Duren¹, A.U. Jackson¹, T.T. Valle², J. Tuomilehto², R.N. Bergman³, F.S. Collins⁴, M. Boehnke¹.* 1) U. Michigan, Ann Arbor, MI; 2) Nat'l Public Health Inst., Helsinki, Finland; 3) U. Southern California, Los Angeles, CA; 4) NHGRI, Bethesda, MD.

Younger age-of-diagnosis (AOD) UK type 2 diabetes (T2D) families showed greater evidence of linkage for T2D compared to older onset T2D families (Frayling et al., 2003). We examined the evidence for linkage in 441 families with mean T2D AOD 55 years of age (mean AOD =47.75.5) and 295 families with T2D AOD > 55 years of age (mean AOD =59.23.1) from the Finland-United States Investigation of NIDDM Genetics (FUSION) study. Compared to individuals in AOD > 55 families, individuals in AOD 55 families had higher weight at age 30 (74.315.4 vs. 69.611.9 kg, $p < .0001$) and higher fasting glucose at study (10.33.4 vs 9.43.1 mmol/l, $p < .0001$). In a genome-wide T2D linkage scan, the strongest evidence for linkage in AOD 55 families was $MLS=2.81$ on chromosome 11q and in AOD > 55 families was $MLS=1.27$ on chromosome 21p. We asked if the number of autosomal regions with LOD scores greater than a given value was larger than expected by chance in the AOD 55 families by randomly choosing 441 families and rerunning the linkage analysis 10000 times. We did not observe a significant enrichment of peaks with $MLS > 2.5$ (1 observed, .59 expected, $p\text{-value} = .49$) or $MLS > 2$ (1 observed, 1.23 expected, $p\text{-value} = .79$), but we did observe more peaks than expected with $MLS > 1.5$ (6 observed, 2.51 expected, $p\text{-value} = .006$, peaks on chromosomes 6q, 7q, 11q, 12p, 14q, 17p). None of the regions had a higher LOD score in the AOD 55 families when compared to the total sample: chromosome 6q ($MLS=1.84$ vs. 2.66), 11q ($MLS=2.81$ vs. 2.98), 14q ($MLS=1.70$ vs. 2.31). These results suggest there is enrichment for detectable genetic effects in AOD 55 families but that no one locus is strongly enriched in the younger T2D onset group.

Hypertriglyceridemic type 2 diabetes and impaired glucose tolerance/impaired fasting glucose subjects were linked to chromosome 2q in a genome-wide scan - The San Antonio Family Heart study - *J. Sung^{1,2}, K.J. Hunt³, H.H.H. Göring¹, L. Almasy¹, S. Cole¹, T. Dyer¹, J.W. MacCluer¹, M.P. Stern³, J. Blangero¹*. 1) Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 2) Department of Preventive Medicine, Kangwon National University, Chuncheon, Korea; 3) Division of Clinical Epidemiology, Department of Medicine, University of Texas Health Science Center, San Antonio, TX.

A search for the genes responsible for type 2 diabetes has been partially successful with some replications. One explanation for the diverse results might be found from properly dissecting the heterogeneous phenotypes. Tryglyceride level is problematic to most diabetic patients, and hard to manage for some even after glycemic control. We performed a genome-wide linkage study to localize possible susceptibility genes influencing hypertriglyceridemic type 2 diabetes. Impaired glucose tolerance (IGT) or impaired fasting glucose (IFG), as well as diabetes in 38 randomly ascertained, extended, Mexican American pedigrees with 961 individuals. Participants were examined at baseline and average 5.1 years later. Diabetes or IGT/IFG was defined by 1) previous diagnosis of diabetes and/or diabetic medication, or 2) exceeding 110 mg/dL of fasting plasma glucose level or 140 mg/dL of postprandial 2-hour glucose level in either of two examinations. There were 351 subjects who met diabetes-related criteria, and 151 of 351 were hypertriglyceridemic ($>2.3\text{mmol/L}$). We performed logistic regression to take the residual of dichotomous traits adjusting for demographic factors, then normalized the residuals. We used multipoint IBD matrices derived from a 10 cM genomic map, and a variance decomposition method (SOLAR). In the final model, dietary factors and medications were considered. Heritability was estimated to be 0.63. The highest multipoint LOD (MLOD) score of 3.49 was found on chromosome 2 at Loc 195 cM for a comparison within the diabetic subjects ($n=351$), and LOD of 2.50 at chromosome 16 Loc 70 cM in total subjects ($n=961$). This chromosome 2 region was proximal to the previous reports for diabetes (245-261 cM). This work was supported by a grant from the NHLBI (P01 HL45522).

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Candidate gene analysis of a T1D susceptibility locus. *T.L. Tabone, G. Morahan.* Genetics & Bioinformatics, The Walter & Eliza Hall Inst, 1G Royal Parade, Parkville, VIC, Australia.

In this study, a novel type 1 diabetes (T1D) susceptibility interval on human chromosome 19p13 was identified. A putative T1D susceptibility gene is proposed to be in linkage disequilibrium to a microsatellite marker, to which a LOD score of 3.3 was obtained from analysis of British multiplex families with T1D.

A positional candidate gene approach was employed to identify the potential causal variant of T1D susceptibility segregating in the multiplex families. Several candidate genes were identified in this region and genomic variants in these genes were sought to test association with T1D susceptibility.

Linkage and linkage/disequilibrium analysis suggest the potential causal variant of T1D in the multiplex families is within a small and genetically well-defined region, facilitating its identification in the near future.

Examination of locational candidate genes in age related macular degeneration. A. Agarwal¹, L.M. Olson², J.R. Gilbert³, N. Schnetz-Boutaud², S. Schmidt³, W.K. Scott³, E. Postel⁴, M.A. Pericak-Vance³, J.L. Haines². 1) Ophthalmology and Visual Sciences, Vanderbilt University, Nashville, TN; 2) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 3) Center for Human Genetics, Duke University, Durham, NC; 4) Duke Eye Center, Duke University, Durham, NC.

Age-Related Macular Degeneration (AMD) is one of the most significant causes of vision loss in the elderly. There is substantial evidence that genes play a critical role in its etiology. As part of our ongoing genetic epidemiological studies of AMD, we participated in a genomic screen of 391 rigorously diagnosed multiplex families with AMD, identifying several chromosomal locations to be further examined, including 1q25-32, 10q25-26, and 14q11-13. Genes within these regions thus represent locational candidates for further study. We tested five genes on 1q (FIBL-6, OCLM, CRB1, OPTC, REN), two genes on 10q (GSTO1, PRDX3), and one gene on 14q (NRL), additionally selected based on their potential functional role in AMD. A mutation in FIBL-6 has been previously proposed as a causal variant for AMD. We tested multiple SNPs in each gene on two independent datasets. The first consisted of 165 families and the second consisted of 401 cases and 159 examined normal controls. We performed both family-based (single locus: Pedigree Disequilibrium Test [PDT]; multilocus: FBAT) and case-control (Chi-square) analyses. 29 SNPs with minor allele frequencies >0.20 were tested in these eight genes. No significant associations were seen in either dataset for any of the 20 SNPs on chromosome 1q, for the two tested SNPs in GSTO1 on chromosome 10q, or the four tested SNPs in NRL on chromosome 14. Two SNPs in PRDX3 (peroxiredoxin 3, an antioxidant protein) gave nominally significant P values ($p=0.05$, 0.04), but only in the case-control analyses and neither survive correction for multiple comparisons. Thus despite positive linkage information and plausible functional importance, none of these polymorphisms appear to represent a significant risk factor for AMD.

Final steps in identification of the X-linked lethal infantile spinal muscular atrophy (XL-SMA) gene. M.E.

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X-linked lethal infantile motor neuron disease (MIM 301830), closely resembles Werdnig-Hoffman disease, with the additional features of early onset or congenital contractures and/or fractures. Fourteen unrelated families have been identified from North and Central America and Western Europe. Of these, nine families have been tested for linkage on the X-chromosome; all families mapped to the same Xp11.3-Xq11.2 region. One family is thought to represent a new mutational event. The cumulative LOD score is 8.71 at $\alpha = 0.0$ at DXS1003. In the past year we have used the ABI Linkage Mapping Set X chromosome markers from the Xp11.3-Xq11.2 region supplemented with additional markers for further linkage analysis. This has not yielded any new recombination events however, new LOD score information has been generated. We are currently using ABI Assay-on-Demand SNPs in this region to further narrow the candidate disease gene interval. Mutation screening through genes in the region has proceeded jointly between University of Miami (UM) and Ludwig Maximilians University (LMU) pursuing complimentary approaches. UM screening has focused on selection and study of candidate disease genes based on presumed protein functions that relate to RNA processing, neuronal or muscle development, cell to cell communication and apoptosis. Growing knowledge concerning the molecular and cellular basis of autosomal SMA is used for selection many of these candidates. At LMU, all cDNAs in the disease gene region are being tested for mutations in XL-SMA samples using high-throughput methodologies. In total, approximately 60 genes within the candidate region have been tested with no mutations found in affected males or carrier females. It is anticipated that we will complete gene screening by October 2004. Our most recent results will be presented. *This study has been generously supported by grants from the MDA, FSMA, Payton's Pals, UM School of Medicine and the Dr. John T. Macdonald Foundation Center for Medical Genetics..*

Linkage heterogeneity in 254 hereditary prostate cancer (HPC) families. *M.D. Badziuch¹, J.L. Stanford², D.M. Friedrichsen², S. Kolb², E.A. Ostrander², M. Janer³, L. Hood³, G.P. Jarvik¹.* 1) University of Washington, Seattle, WA; 2) Fred Hutchinson Cancer Research Center, Seattle, WA; 3) Institute for Systems Biology, Seattle, WA.

The 254 Seattle/PROGRESS HPC families (mean cases/family= 4.45) have previously undergone a 10 cM genome scan by 2-point lod and multipoint NPL analyses. Additional analyses were undertaken to examine potential linkage heterogeneity (het). Model-based 3-point lod scores were assessed for het by the alpha-test. Using the sex specific Marshfield map, the most significant het was with D7S2212/D7S820 at chromosome (chr) 7q21 (lod<0, hlod= 1.59, p-het= 0.007) under a recessive model; the location of a proposed HPC locus (Friedrichsen, 2004). Under a dominant model, D11S2371/D11S2002, chr 11q13-14, gave hlod= 1.58, p-het= 0.01. The differences (d) between pairs of affected brothers' ages or Gleason score (GS) were examined for associations with multi-marker ibd allele sharing, a model free approach (SAGE LODPAL). A >60 cM span on chr 9q21-33 had age-d effects (p < 0.01), peaking at D9S930/D9S934 (lod= 3.07, p= 0.0002). Chr 11q23-24 gave an age-d lod of 2.85, p= 0.0003. GS was known for 266 brother pairs in 94 families and showed d effects on chrs, 2q37 (lod= 2.11, p=0.002), 8q21-23 (2.23, 0.002) and 18q21 (1.81, 0.004). Small d predicted linkage in most cases. Some markers yielding these results were previously associated with number-of-sibs-affected (GAAT1A4/D8S1132) by Goddard, et al (2001) and with GS (D9S930/D9S934) by Witte, et al (2000). As studies suggest many HPC loci, methods robust to het and consistent linkage signals among datasets should be pursued. Small d may be a useful covariate in HPC linkage.

Replication of Linkage and Refinement of *IGAN1* Locus. I. Beerman^{1,2}, A. Gharavi^{1,2}, G. Frasca⁴, A. Amoroso⁴, D. Pirulli⁴, C. N.-Williams^{1,2}, S. Woodford⁵, R. Wyatt⁵, B. Julian⁶, G. Giggheri⁷, F. Scolari⁸, R. Lifton^{1,2,3}. 1) Genetics, Yale, New Haven, CT; 2) HHMI, Yale, New Haven, CT; 3) Medicine, Yale, New Haven, CT; 4) Bologna, Italy; 5) Memphis, TN; 6) Birmingham, AL; 7) Genoa, Italy; 8) Brescia, Italy.

IgA nephropathy (IgAN) is the leading cause of glomerulonephritis worldwide. Though IgAN is a significant contributor to renal failure, little is known about the pathogenesis and there are no effective treatments for this disease.

Familial segregation and ethnic clustering suggest a genetic contribution to IgAN. Genome-wide linkage analysis of 30 multiplex kindreds demonstrated that familial IgAN links to a 6 cM region (*IGAN1*) on 6q22-23. Parametric analysis using a model of dominant transmission with 75% penetrance yielded a peak lod score of 5.6 with 60% of kindreds linked to *IGAN1*. (*Nature Genetics*, 2000 **26**:354-357)

To replicate these findings, we studied an independent cohort of 16 IgAN kindreds (40 affecteds) applying the same phenotypic criteria as previously. We genotyped these kindreds for 25 microsatellite markers encompassing the lod-1 interval. Using the parameters from the initial study, these families yielded a lod score of 1.5 at the initial lod score peak with an alpha of 0.45 (point wise $p < 0.005$) achieving published criteria for significant replication. The significance was also validated by 1000 simulations, yielding an empiric p-value of 0.006.

For kindreds linked to *IGAN1*, identification of additional affected members has the capacity to further test the hypothesis of linkage. We identified a 5th cousin in one such kindred with biopsy documented IgAN. This cousin is separated by 11 meiotic events from the closest affected individuals in the pedigree and shares an 8 cM haplotype with the previously linked haplotype. This kindred alone now contributes a lod score of 4.4 and reduces the *IGAN1* minimal recombinant interval to 2 cM. Combining the data from all 46 IgAN families, multipoint linkage analysis at *IGAN1* yields a lod score of 9.3 ($=0.48$). The present findings confirm linkage and refine the lod-1 interval for IgAN to a 4.4 cM segment spanning ~4.9Mb.

A genomic screen for amyotrophic lateral sclerosis identifies several novel candidate regions of linkage. *Y. Bradford*¹, *T. Siddique*², *R.H. Brown*³, *D. McKenna-Yasik*³, *N. Siddique*², *W.-Y. Hung*², *B. Hosler*³, *S. West*⁴, *M.A. Pericak-Vance*⁴, *J.L. Haines*¹. 1) Center for Human Genetics Research, Vanderbilt Univ, Nashville, TN; 2) Davee Department of Neurology, Northwestern Univ, Chicago, IL; 3) Day Neuromuscular Laboratory, Massachusetts General Hospital, Boston, MA; 4) Center for Human Genetics, Duke Univ., Durham, NC.

Amyotrophic lateral sclerosis (ALS; Lou Gehrig disease) is an irreversible degenerative neuromuscular disease usually resulting in death within 5 years of (typically) mid-life onset. While some large autosomal dominant adult onset and autosomal recessive juvenile onset pedigrees have been identified, mutations in only two genes (SOD1, Alsin) have been definitively shown to cause ALS. The severe course and later onset of ALS makes finding multiplex families difficult. Despite this, we have assembled a dataset of 54 adult onset multiplex families with 145 sampled affected individuals and 122 total affected relative pairs. All families were screened and were negative for mutations in the SOD1 gene. 300 microsatellite markers (~11 cM average spacing) were genotyped through these families. Parametric two-point analyses were performed under a dominant and a recessive model and the maximal heterogeneity lod scores across both models were calculated. Multipoint non-parametric scores (lod*) were also calculated. 12 markers, representing seven chromosomal regions (1p, 7p, 8q, 9p, 17p, 18p, 19q), generated two-point lod scores >1.0 in the overall dataset. Four of these regions (7p, 8q, 18p, 19q) also generated lod* scores >1.0. Of particular interest are the results on chromosome 19q, where the peak multipoint lod score was 2.0. These data suggest that further locus heterogeneity in ALS.

Genome-wide Linkage Analysis of Recurrent, Early-Onset Major Depression and Anxiety Disorders in Extended Utah Pedigrees. *N.J. Camp*^{1,2}, *M.R. Lowry*³, *R.L. Richards*⁴, *A.M. Plenk*⁵, *D.C. Hughes*¹, *L.A. Cannon-Albright*^{1,2}. 1) Genetic Research, Intermountain Health Care, Salt Lake City, UT; 2) Genetic Epidemiology, Univ Utah, UT; 3) Department of Psychiatry, Univ Utah, UT; 4) Private Practice, UT; 5) The Children's Center, UT.

Major depressive disorder (MDD) is a common and clinically heterogeneous disorder, often found comorbid with other disorders. We studied recurrent, early-onset MDD (MDD-RE) and anxiety disorders in combination in an attempt to define powerful phenotypes for genetic study. We used 87 extended Utah pedigrees ascertained for family history of MDD to investigate linkage to 3 phenotypes: 'MDD-RE'; 'MDD-RE or anxiety' and 'MDD-RE and anxiety', where in the latter definition the disorders must appear comorbid within the same individual. Pedigrees ranged in size from 2 to 6 generations, and contained between 3 and 42 individuals affected with MDD or anxiety. We identified 5 regions on chromosomes 3centr, 4q, 7p, 15q and 18q, with all except the centromeric region on chromosome 3 representing replication findings. In our analyses, the regions on chromosomes 4q and 15q were identified by sex-specific analyses. The best linkage evidence was found for a novel locus at 3p12.3-q12.3 (LOD = 3.88 for 'MDD-RE or anxiety') and at 18q21.33-q22.2 (LOD = 3.75, 'MDD-RE and anxiety') which is well-established as a susceptibility locus for bipolar disorder. Chromosomes 4q and 7p have previously been implicated as loci involved in neuroticism (4q and 7p) and panic disorder (7p), and the region on 15q for MDD-RE. Using linked pedigrees we localized the 3centr and 18q regions to 9.8 and 12.2 cM, respectively, with potential for further localization with the addition of markers in specific linked pedigrees. Our success in replication and substantial novel finding on 3centr illustrates the utility of extended pedigrees for common disorders, such as MDD. Further, it lends weight to the hypothesis that MDD and anxiety disorders have over-lapping genetic etiologies, and suggests that not only early-onset and recurrence, but comorbid diagnoses, may be useful in defining more genetically homogeneous forms of MDD phenotypes for linkage mapping.

Linkage evidence for Cutaneous Malignant Melanoma to 1p22 in Utah Pedigrees. *L.A. Cannon-Albright¹, J.M. Farnham¹, S. Leachman², N.J. Camp¹.* 1) Genetic Epidemiology, University of Utah School of Medicine, UTAH; 2) Dermatology, Huntsman Cancer Institute, University of Utah, UTAH.

The Utah extended pedigree resource for cutaneous malignant melanoma (CMM) currently comprises 24 pedigrees, ten of which were part of the Utah resource used to localize 9p21 and isolate p16. Pedigrees range in size from 3-8 generations and contained between 2-19 CMM cases (total 150). These 24 pedigrees were used to investigate the proposed CMM locus at chromosome 1p22. Our phenotype was CMM, confirmed by the Utah Cancer Registry data in the Utah Population Database (UPDB) or by medical records. Our genotyping consisted of 4 microsatellites spanning 9cM of the previously reported multipoint peak. We chose two genetic models; an affecteds-only age model, with age-dependent penetrances as determined from the original Utah resource, and a general affecteds-only dominant model. We performed multipoint linkage analysis using MCLINK, a Markov chain Monte Carlo method incorporating block Gibbs updating, which allows analysis of extended pedigrees. To combat the problem of intra-familial heterogeneity inherent in the analysis of extended pedigrees, we also performed the analysis on pedigree-splits. We used an unbiased splitting algorithm, prior to genotyping data, which iteratively removes founders from the pedigrees thus reducing the number of generations analyzed. The global HLODs were not supportive of linkage to 1p22, with the max HLOD found being 0.118 at D1S2671, using the age model and pedigree-splits with a maximum of 3 generations. We were able to locate 4 pedigree-splits that obtained pedigree LODs of between 0.528-0.867, with 3 or 4 CMM cases sharing a segregating haplotype. The average age at diagnosis of CMM was 46.5 yrs in haplotype carriers, but this was not significantly different from 52.0 yrs (all pedigrees) or 51.9 yrs (all CMM in the UPDB). No p16 mutations have been found in these 4 1p22-linked pedigrees. In conclusion, there is little evidence to support the 1p22 linkage in the 24 Utah pedigrees analyzed here. At best, the 4 pedigrees with nominal individual linkage evidence may be of use for screening for putative variants in the region, if any are discovered.

Progressive myoclonus epilepsy with polyglucosans (Lafora disease): evidence for a third locus. *E.M. Chan¹, S. Omer², M. Ahmed³, L.R. Bridges⁴, C. Bennett³, S.W. Scherer¹, B.A. Minassian^{1,5}*. 1) Program in Genetics & Genomic Biology, The Hospital for Sick Children, Toronto, Canada; 2) Department of Neurology, St. George's Hospital, London, UK; 3) Department of Clinical Genetics, St. Jame's University Hospital, Leeds, UK; 4) Department of Pathology, University of Leeds, Leeds, UK; 5) Division of Neurology, The Hospital for Sick Children, Toronto, Canada.

Lafora disease (LD) is an autosomal recessive disorder and the most severe form of teenage-onset progressive myoclonus epilepsy. LD is a unique type of glycogenosis with accumulation of a ER-associated starch-like polyglucosans forming Lafora bodies. A homogeneous clinical presentation exists despite the presence of genetic heterogeneity. Recessive mutations in either of two genes cause the syndrome, *EPM2A*, encoding a dual-specificity phosphatase with a carbohydrate-binding domain (named laforin), and *EPM2B*, encoding a putative E3 ubiquitin ligase (named malin). In total, 88% of our collection of LD families can now be accounted for by mutations in *EPM2A* (48%) and *EPM2B* (40%). We describe a family with three members affected with classic LD. Affected members of this family have no mutations in the coding regions and flanking intronic sequences of either *EPM2A* or *EPM2B*. Linkage and haplotype analyses exclude both loci from causative involvement in the disease in this family. These results indicate that a third LD gene exists. Its identification will be crucial in understanding the cellular pathway underlying polyglucosan formation. In addition, the description of additional genetic heterogeneity in LD will improve patient diagnosis, prenatal diagnosis and genetic counseling for this severe form of epilepsy.

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A genome-wide linkage analysis in a large pedigree with Ehlers-Danlos syndrome, hypermobility type. *A. de Paepe, F. Malfait, P. Coucke.* Center for Medical Genetics, Ghent University Hospital, De Pintelaan 185, B-9000 Ghent, Belgium.

The Ehlers-Danlos syndrome (EDS) is a clinically and genetically heterogeneous group of heritable connective tissue disorders that is characterized by skin hyperextensibility, joint hypermobility and generalized connective tissue fragility. The most recent classification (Villefranche Nosology, 1997) recognizes six subtypes, of which the hypermobility type (former type III) is the most frequent one. This is an autosomal dominant disorder that is characterized by generalized joint hypermobility, repetitive dislocations of large and small joints, chronic musculoskeletal pain, and mild involvement of the skin with soft skin and mildly dilated scars. Except for a mutation in COL3A1, encoding collagen type III, and some mutations in TNXB, encoding tenascin-X, no causal mutations have been identified yet. We have performed linkage analysis in a three-generation pedigree of Belgian origin with EDS, hypermobility type. This family counts at least 13 affected and 8 non-affected members. Although there was some variability in the severity of the phenotype, all affected members clearly fulfilled the criteria for EDS, hypermobility type. In this family we excluded linkage to the COL1A1, COL1A2, COL3A1, COL5A1 and COL5A2 gene, the genes that are involved in other autosomal dominant types of EDS. We performed a genome-wide segregation analysis and established linkage to a 5.5cM region with a maximum LOD-score of 3.32. Ongoing studies are planned to confirm the region in other families, and mutational analysis of the strongest candidate genes within the minimal region will be performed.

Homozygosity mapping in mediterranean families identifies a novel Charcot-Marie-Tooth neuropathy (CMT4H) on chromosome 12p11.21-q13.11. A. DeSandre-Giovannoli^{1,2}, V. Delague¹, T. Hamadouche^{1,3}, E. Chouery⁴, I.

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Charcot-Marie-Tooth (CMT) neuropathies are a group of clinically and genetically heterogeneous hereditary motor and sensory neuropathies (HMSN); mainly characterized by muscle weakness and wasting, foot deformities and electrophysiological as well as histological changes. CMT1 is defined by a reduction of nerve conduction velocities with demyelinated fibers and CMT2 by a slight or absent reduction of nerve conduction velocities (NCVs) together with loss of large myelinated fibers and axonal degeneration. Homozygosity mapping in two consanguineous Mediterranean families from Lebanon and Algeria affected with severe autosomal recessive demyelinating CMT, evidenced linkage to chromosome 12p11.21-q13.11 in a 11.5 cM region between microsatellite markers D12S1648 and D12S1661. A maximum pairwise LOD score value (Z_{max}) of 6.97 at $p = 0.001$ was obtained for marker D12S345. Among the 90 genes identified in the candidate region, the entire coding region of two potential candidate genes, Contactin 1 (*CNTN1*) and Peripherin (*PRPH*), has been tested for the presence of mutations. Although their functions were suggestive of an implication in CMT, no disease causing mutations were identified in either gene, and *PRPH* has definitely been excluded as the causative gene for CMT4H, since it has been mapped outside the candidate interval by further genotyping. We propose to add this phenotype and locus to the expanding CMT classification, as being the CMT4H. Besides, we will present our clinical and molecular explorations regarding this severe neuropathy.

Concordance and call rate assessment of the Affymetrix 10K SNP array. *K.R. Ewen-White¹, S.C. Herbert¹, M. Papoulis¹, K. Siemering¹, G.W. Montgomery², N.G. Martin², S.M. Forrest¹.* 1) Australian Genome Research Facility, 1G Royal Parade Parkville Victoria 3050, Australia; 2) Queensland Institute of Medical Research Post Office, Royal Brisbane Hospital 300 Herston Road Brisbane 4029, Australia.

The Australian Genome Research Facility has recently installed the latest Affymetrix system incorporating the GS3000 high density scanner allowing processing of 10K and 100K SNP chips as well as all Affymetrix gene expression chips. The 10K SNP chip contains 11,555 SNPs giving an average genetic gap distance of 0.32cM and average estimated heterozygosity of 0.35. Studies suggest that around 3 SNP markers are equivalent to 1 microsatellite marker implying the 10K chip will provide significantly greater information than a 10cM microsatellite scan and at a much cheaper price of < \$0.10/GT. Published results of complex trait mapping have demonstrated that the 10K chip enables increased resolution of LOD peaks as well as highlighting additional minor peaks. These genotyping improvements have assisted in defining new regions as well as decreasing fine mapping requirements. The 10K chip is also being used to detect chromosomal deletions & amplifications up to 10 times smaller than have been found with spotted microarrays and comparative genomic hybridisation (CGH).

The AGRF have assessed the 10K chip for reproducibility measuring concordance between replicate samples and between monozygotic twins. The effect of altering call zone boundaries on call rate and accuracy has been determined. The consistency of SNP failures between individual arrays has been assessed and the effect of DNA quality and preparation methods on final call rates has been measured.

Search for endometriosis susceptibility genes in Puerto Rico. *I. Flores*¹, *D.M. Mandal*², *J.E. Bailey-Wilson*³. 1) Department of Microbiology, Ponce School of Medicine, Ponce, PR; 2) Dept. of Genetics, LSU Health Sciences Center, New Orleans, LA; 3) Inherited Disease Research Branch, NHGRI/NIH, Baltimore, MD.

The causes of endometriosis are unknown although the evidence of genetic susceptibility is extensive. The main goal of the present study is to provide the genetic basis of endometriosis in the population in Puerto Rico. Thirty-two families with two or more affected members were recruited from the island of Puerto Rico. Genotyping data were obtained on 18 markers in chromosome 10 candidate regions. The data have been checked for Mendelian inconsistencies using the program GCONVERT/SIB-PAIR. Significant allelic association was observed at the empiric p-value of 0.0038 at one of the candidate regions. The mode of inheritance of endometriosis is not known. Therefore, the LOD score was calculated assuming two simple models, dominant and recessive, each with an arbitrary 50% penetrance in gene-carriers and 5% penetrance in non-gene carriers. We used Genehunter-Plus to compute multipoint parametric LOD scores using these two models, as well as multipoint non-parametric NPL statistics and the Kong and Cox allele-sharing LOD score. Affected only analysis was performed by coding the unaffected women as unknown in the analysis. Assuming an autosomal dominant mode of inheritance, the highest LOD score obtained was 1.75 and under the autosomal recessive model, the highest LOD score obtained was 1.26. We will be extending these analyses by using models that incorporate age through multiple liability classes.

Continuing analysis of a prostate cancer susceptibility locus on 7q11-21 in Jewish families. *D.M. Friedrichsen¹, J.L. Stanford², M. Janer³, K. Deutsch³, S. Kolb², M.D. Badzioch⁴, G.P. Jarvik⁴, L. Hood³, E.A. Ostrander¹.* 1) Dept Human Biol; 2) Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 3) Institute for Systems Biology, Seattle, WA; 4) Department of Medicine, Division of Medical Genetics, University of Washington, Seattle, WA.

An estimated 220,900 men will be diagnosed with prostate cancer (PC), and 28,900 deaths will be attributed to the disease in the United States this year. Both epidemiological studies and segregation analyses confirm the existence of a genetic component to PC etiology, with an estimated 5-10% of all PC and 43% of early onset (55 years) disease being attributed to an inherited susceptibility. Recently, ten genome-wide scans have been published, analyzing 1,730 hereditary PC (HCP) families. The results of these studies confirm that a substantial amount of genetic heterogeneity exists in HPC.

One approach to reduce locus heterogeneity is to analyze Jewish families, which represent a more well-defined and genetically homogenous population. In our previously reported combined genome-wide scan of 36 Jewish HPC families from the Seattle-based PROGRESS study and Johns Hopkins University, we identified a region of significant linkage on chromosome 7q11-21, with an empirical P value of 0.006. Further resolution of the 7q11-21 locus in 18 Jewish PROGRESS families indicates that the minimal recombination region is 5.7 cM and contains approximately 20 RefSeq genes. This region expands to 29.2 cM and would contain over 100 RefSeq genes if three recombination events are used to define either boundary. Both SNPs and microsatellites are being evaluated in order to identify a potential founder haplotype. We have screened over 200 amplicons thus far and no obvious mutations have been found. This study highlights the utility of analyzing defined sets of families with a common origin for reducing locus heterogeneity problems associated with studying complex traits.

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Linkage analysis allowing for heterogeneity and marker informativity. *T.A. Greenwood, N.J. Schork.* Dept Psychiatry, Univ California, San Diego, La Jolla, CA.

Locus heterogeneity is known to plague the identification of disease susceptibility loci in linkage mapping contexts of complex traits and diseases. Even within a field center to be used for sampling individuals for a study the family sets are likely to contain individuals with varying genetic backgrounds and genetic susceptibility profiles. We have developed novel mixture models that accommodate both locus heterogeneity and marker informativity and have applied it to large data sets. Our results provide information on not only regions of the genome likely to harbor susceptibility loci but also information on the degree to which heterogeneity influences the linkage signal at that locus. Ultimately, we find that linkage analyses that allow for heterogeneity can complement traditional techniques that do not. Our methods and analyses complement approaches taking advantage of context-specific linkage analysis, since the proposed heterogeneity models do not necessarily make assumptions about the nature of the factors contributing to and differentiating families showing linkage from those that do not (but can, if particular covariates are specified). Thus, our proposed models can be used to address questions about the ultimate comparability and combinability of data collected in different racial groups and field centers.

A comprehensive search for genetic susceptibility to MS on the X chromosome. *B.M. Herrera³, H. Armstrong², D. Dyment³, Z. Cader³, G.C. De luca³, M.R. Lincoln³, S. Orton³, C. Willer³, K. Morrison³, G.C. Ebers¹.* 1) Department of Clinical Neurology, Radcliffe Infirmary, Oxford University, Oxford, UK; 2) Dept. of Clinical Neurosciences, London Health Sciences Center, London Ontario, Canada; 3) Wellcome Trust Centre for Human Genetics, Oxford University, Oxford, UK.

Genetic and epidemiological studies have shown the importance of genes in MS susceptibility. Only one association has been consistently replicated in genetic studies: the HLA-DRB15 on chromosome 6p21. Linkage and association studies have identified candidate regions on the X chromosome. An unexplained female to male ratio of 2:1, is present in all studies. Observations from affected sib-pairs and half-sibs demonstrate a maternal parent of origin effect. We performed a genome scan of the X chromosome, in two datasets; the first consists of 552 affected sib-pairs, where we genotyped 22 X chromosome microsatellite markers. A lod score of 1.72 was found for marker DXS1683 in 219 of these sib-pairs, when stratified by the presence of DRB15 (Dyment et al 2004). In a second sample of 158 families with affected pairs of aunt-uncle/niece-nephews (AUNN), we genotyped 18 X-chromosome markers. The structure of the AUNN dataset allowed us to divide families by the sex of the carrier parent, so we were able to investigate a potential parent of origin effect in maternal and paternal families. We found no evidence for linkage within these datasets and were able to exclude linkage at $\lambda=1.33$. This study represents an exhaustive scrutiny of the X chromosome as a region involved in susceptibility to MS. Despite many suggestions from previous studies, we were not able to confirm previous observations of linkage to this region. This study shows that the X chromosome is not responsible for the observed maternal effect in MS susceptibility which is double than for paternal risk.

Genome Wide Search for Sarcoidosis Susceptibility Genes in African Americans. *S. Iyengar*¹, *C. Gray-McGuire*¹, *R.C. Elston*¹, *B. Rybicki*², *M. Iannuzzi*³, *for the SAGA consortium*³. 1) Dept Epid/Biostat, Case Western Reserve Univ, Cleveland, OH; 2) Department of Biostatistics and Research Epidemiology, Henry Ford Health System, Detroit, MI; 3) Division of Pulmonary, Critical Care, and Sleep Medicine, Mt. Sinai School of Medicine, New York, NY.

Sarcoidosis is a systemic granulomatous inflammatory disease of unknown origin that can involve any organ. The current thinking is that the granulomatous reaction characteristic of sarcoidosis occurs as a result of an immune dysregulation following an initial immune response to an inciting environmental agent in a genetically susceptible host. In the United States, African Americans are more commonly affected with sarcoidosis and suffer greater morbidity than Caucasians. We searched for sarcoidosis susceptibility loci by conducting a genome-wide, sibpair multi-point linkage analysis in 229 African-American families ascertained through two or more sibs with a history of sarcoidosis. Using the Haseman-Elston regression technique, linkage peaks with asymptotic p values less than 0.05 were identified on chromosomes 1p22, 2p25, 5p15-13, 5q11, 5q35, 9q34, 11p15 and 20q13 with the most prominent peak at D5S2500 on chromosome 5q11 (p=0.0005). We found agreement for linkage with the previously reported genome scan of a German population at chromosomes 1p, 3p and 9q. Although we found no evidence for linkage to the MHC region of chromosome 6p in our initial genome scan, an additional marker added to this region, D6S1701, showed marginally significant linkage to sarcoidosis (p=0.04). This region on chromosome 6 has previously shown association in case control studies. Our results validate the case control studies. Based on the multiple suggestive regions for linkage found in our study population, it is likely that more than one gene influences sarcoidosis susceptibility in African Americans. Fine mapping of the linked regions, particularly on chromosome 5q, should help refine linkage signals and guide further sarcoidosis candidate gene investigation.

Genomewide analyses demonstrate that novel loci predispose to drusen formation. *G. Jun¹, B.E.K. Klein², R. Klein², K. Fox¹, C. Millard¹, J. Humphrey¹, K. Russo¹, K.E. Lee², R.C. Elston¹, S.K. Iyengar¹.* 1) Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH; 2) Ophthalmology and Visual Sciences, University of Wisconsin Medical School, Madison, WI.

The accumulation of lipoproteinaceous deposits (drusen) is the earliest clinical hallmark of age-related maculopathy (ARM), a multifactorial degenerative eye disease. We hypothesized that examination of size and type of drusen would give better insights into early ARM pathogenesis. We performed genomewide model-free linkage analysis using two semi-quantitative drusen traits, size and type using two sets of data: (1) 325 individuals (225 sib pairs) in 102 families from the Beaver Dam Eye Study (BDES), and (2) 297 individuals (346 sib pairs) from 34 extended families in the Family-based Age-Related Maculopathy Study (FARMS). We found evidence of linkage on chromosome 19q13.31 with size in both BDES ($p=0.029$) and FARMS ($p=0.0013$). In BDES, type ($p=0.019$) and size ($p=0.026$) showed evidence at 3p24.3, and type only at 3q25.1 ($p=0.014$). In FARMS, size showed evidence at 5q33.3 ($p=0.0021$) and 16p13.13 ($p=0.0015$), and type at 21q21.2 ($p=0.0070$). The FARMS dataset which was ascertained for severe disease gave more distinctive linkage signals. We utilized APOE genotypes as a covariate in a multipoint sibpair linkage analysis, since the chromosome 19 locus provided good support of linkage. We observed a small decrease in the linkage signal using the 2/4 genotype (reference 3/3) with size (p -values decreased from 0.0357 to 0.0719 in BDES and from 0.0087 to 0.0168 in FARMS). This result demonstrated that APOE gene did not fully account for the 19q13.31 signal. This suggests that a novel locus, close to APOE, is responsible for drusen formation. APOE has previously been associated with age related macular degeneration (AMD) in case control studies, but genome scans for AMD have been unable to detect any linkage on 19q13. Our results show that APOE effects may be mediated early in the ARM to AMD process, and thus may not be detected by standard genome scans for more severe disease.

A 10K SNP Genomic Screen in Extended Late Onset Alzheimer Disease Families. *S.J. Kang¹, C.S. Haynes¹, J.L. Haines², E.R. Martin¹, D.A. Stephan³, M.A. Pericak-Vance¹.* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 3) The Translational Genomics Research Institute, Phoenix, AZ.

Alzheimer disease (AD) is a common, genetically complex disease whose etiology is not yet fully described. Although four genes have been identified and several other loci have been proposed, loci for a number of large families have not yet been identified. We have genotyped over 10,000 SNPs across the genome in 11 families using the Affymetrix 10K SNP chip. These multi-generational Caucasian families contain between 5 to 8 sampled individuals with AD, for a total of 69 cases. The mean number of sampled individuals per family was 10.538 (means.d.) with a minimum of 6 and maximum of 18. These families showed no evidence of linkage to previously reported linkage regions on chromosomes 2, 9, 10, and 12 and had no known evidence of mutations in APP, PS1, or PS2. Two-point parametric linkage analysis using a dominant model was performed using FASTLINK and HOMOG. Information extraction was substantially increased over our previous 8 cM microsatellite screen, demonstrating the power of the dense SNP mapping approach. The maximum LOD score across all SNPs was 4.36 for RS275838 (78.03cM) on chromosome 19, confirming the effect of APOE (71.55cM), an established risk factor for AD. LOD scores over 2.0 were obtained on chromosomes 1, 2, 3, 4, 5, 7, 8, 18, 19, 20 and 21. Aside from APOE, LOD scores greater than 2.5 were obtained on chromosome 2 (129.35cM), chromosome 4 (152.88cM), chromosome 5 (112.61cM) and chromosome 8 (74.93cM), suggesting several novel locations for further analysis (The previously reported linkage region on chromosome 2 was at 210.26cM). Multi-point analyses are ongoing as are conditional analyses controlling for APOE genotype.

Confirmation, Localization and Genetic Association of SLEH1 on 11q14 in African-American Multiplex SLE Pedigrees with Hemolytic Anemia. *J.A. Kelly¹, S. Shadfar¹, K.M. Kaufman^{1,3}, T.J. Aberle¹, G.R. Bruner¹, J.B. Harley^{1,2,3}.* 1) Dept Arthritis/Immunology, Oklahoma Medical Res Fndn, Oklahoma City, OK; 2) University of Oklahoma, Oklahoma City, OK; 3) US Dept of Veterans Affairs Medical Center, Oklahoma City, OK.

The SLE susceptibility locus, SLEH1, was first identified on 11q14 (D11S2002; LOD=4.7) in 16 African-American (AA) multiplex pedigrees that contained at least one individual affected with both SLE and hemolytic anemia (PNAS 99(18):11766-11771, 2002). In an effort to confirm this finding, we identified an additional 13 African-American pedigrees multiplex for SLE with hemolytic anemia in one or more SLE affected. The additional 13 pedigrees confirmed linkage to SLEH1 (LOD=2.0 at D11S2002). To evaluate the region for possible genetic association, we typed 112 SNPs spanning 74.3-85 Mb on chromosome 11q14-21 (average intermarker distance=109 kb; range: 248 bp-1.3 Mb) in 40 AA pedigrees linked to D11S2002. One SNP showed a weak association using TDT ($p=0.004$), PDT ($p=0.04$) and FBAT ($p=0.003$). Fine mapping of an additional 19 SNPs around this SNP produced 6 SNPs that showed family based association (at the $p=0.05$ level). In addition, two SNPs showed significantly different allele frequencies between 40 cases and 46 controls ($2=4.66$, $p=0.03$). For one of the two SNPs, a more significant difference was observed in the allele frequencies between cases and controls when the cases selected had hemolytic anemia ($2=9.31$, $p=0.002$). More SNPs are currently being typed in the neighborhood to define the LD and to help determine whether the possible association signal detected is robust. These results provide further evidence demonstrating that SLEH1 is a strong and reproducible SLE susceptibility locus. The chromosomal locus originally spanning 10 cM is now confirmed and has been further localized to a 2-3 Mb region on chromosome 11q14-21.

Clinical features of a consanguineous Pakistani family with autosomal recessive cone rod dystrophy (CORD8) and refinement of the disease locus (1q23-q24). *S. Khaliq, K. Anwar, A. Abid, M. Ismail, A. Hameed, S.Q. Mehdi.* Biomedical and Genetic Engineering Laboratories, Islamabad, Pakistan.

Cone-rod dystrophies (CRD) are a form of inherited retinal dystrophies which characteristically lead to early impairment of vision due to the simultaneous involvement of both cone and rod photoreceptor cells. Several loci/genes have so far been identified for CRD on various chromosomes including the CORD8 locus identified for a Pakistani family. This locus was mapped on chromosome 1q12-q24, consisting of a very large critical disease region of 21cM, which has been further refined in this study. All affected members of this family underwent clinical examination to determine the nature of the cone-rod dystrophy phenotype associated with this locus (CORD8). Mutation screening of important candidate genes GNAT2, HPRP3, SM4A, CRABP2 and KCNJ10 was carried out and many more microsatellite markers, recently available on database, were analysed to narrow down the disease region. All affected individuals in this family suffered from bilateral cone rod dystrophy with autosomal recessive mode of inheritance. Characteristically all patients have photophobia, marked macular degeneration, attenuated arterioles and pigment epithelium disturbance. No disease-associated mutation was found in any of the candidate genes within the CORD8 locus. Analysis of recently identified microsatellite markers within the previously reported region showed heterozygosity with some of the new markers leading to a refinement of the disease region. This combination of ocular defects suggests a typical cone rod dystrophy phenotype that could be due to a mutation in a novel gene located within the CORD8 locus. Further we have narrowed down the previously reported disease region from 21cM to 11.53cM by analyzing newly available markers and candidate genes in this region.

Mapping the GLC1G locus for Primary Open-Angle Glaucoma (POAG) in an Oregon family of Dutch origin.

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Glaucoma is the second leading cause of blindness worldwide. Six loci and two genes have been mapped for the most common form, primary open-angle glaucoma (POAG), and are designated GLC1A, GLC1B, GLC1C, GLC1D, GLC1E and GLC1F. Myocillin mutations are associated with GLC1A and optineurin mutations are associated with GLC1E. We conducted a genome wide search for a seventh POAG locus, GLC1G, in a large Oregon family of Dutch origin with 92 members and 14 affected individuals, using microsatellite marker genotyping.

We narrowed the search to chromosome 5 and current fine-mapping strategies involve combining data from seven microsatellite markers and sequencing data from 24 single nucleotide polymorphisms (SNPs) located between 104.4 Mb and 111.2 Mb. We use the UCSC Genome Bioinformatics database, assembly July 2003, to determine the chromosomal positions of microsatellite markers and SNPs. We have identified a disease haplotype that spans 3.8 Mb between D5S2084 and D5S492. Mutational analysis of candidate genes is now in progress.

A major gene for keratoconus in Ecuador does not map to any of the known keratoconus loci. *R.A. Lewis¹, M. Gajecka², D. Winters², A. Molinari³, J.A. Pitarque³, M.H. Chahrour¹, S.M. Leal¹, B.A. Bejjani^{2,4}.* 1) Baylor College of Medicine, Houston, TX; 2) Washington State University, Spokane; 3) Hospital Metropolitano, Quito, Ecuador; 4) Sacred Heart Medical Center, Spokane, WA.

Keratoconus (KC) is a non-inflammatory thinning and anterior protrusion of the cornea that results in steepening and distortion of the cornea, altered refractive powers, and altered visual acuity. Although both genetic and non-genetic factors have been associated with KC, its molecular basis is still elusive. We identified an Ecuadorian cohort in which KC without other ocular or systemic features is transmitted as an autosomal dominant trait with incomplete penetrance. To date, we have examined, collected blood, and purified DNA from 148 individuals from 22 multiplex families with KC. Subjects were diagnosed clinically with KC by slit lamp examination and corneal topography. We excluded previously assigned KC loci on chromosomes 3, 15, 16, and 20 by linkage analysis and initiated a genome-wide screen for a KC locus. We genotyped 120 individuals with fluorescent markers with an average spacing of 10 cM spanning chromosomes 3, 4, 15, 16, 19, 20, 21, and 22 and sequenced *VSX1* coding exons in 18 KC individuals from 18 families and two control Ecuadorian individuals. KC in our families is not linked to any of the previously defined KC loci. *VSX1* was excluded as a candidate for KC in this population by sequencing of the coding exons and the exon-intron junctions. We found no evidence for linkage between KC and the markers tested.

Comprehensive genetic linkage analysis of a late-onset Alzheimer disease locus on chromosome 12. X. Liang¹, N. Schnetz-Boutaud¹, S.J. Kenealy¹, K.C. Stanton¹, T.N. Rugless¹, L. Jiang¹, J. Bartlett¹, W.K. Scott², J.R. Gilbert², M.A. Pericak-Vance², J.L. Haines¹. 1) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 2) Ctr for Human Genetics, Duke University, Durham, NC.

Alzheimer disease (AD) is a progressive neurodegenerative disorder of later life with a complex etiology with a strong genetic component. ApoE is the only gene universally accepted as an important risk factor for late-onset AD (LOAD). However, more than 50% of AD cases do not carry ApoE 4 allele, suggesting that other genetic risk factors must exist. Several genomic screens have suggested that a region between chromosome 12p13 and 12q22 is a potential position for an additional locus underlying the susceptibility of AD. However, localization of this locus has been difficult. We hypothesized that some of this difficulty arises from the genetic heterogeneity underlying AD.

To test this hypothesis, we performed a 74 cM microsatellite marker screen on chromosome 12 with 15 markers in 585 multiplex families consisting of 994 affected sibpairs and 213 other affected relative pairs. Analyses across the entire dataset did not reveal significant evidence of linkage. However, suggestive linkage was observed in several more homogeneous subgroups.

In the 91 families where no affected individuals have an ApoE-4 allele, the HLOD is 1.55 at D12S1042, suggesting that ApoE and the chromosome 12 locus might have independent effects. We further examined the linkage data considering the proposed linkages to chromosome 9 (D9S741) and 10 (VR22 gene). Microsatellite marker D12S1701 generated an HLOD score of 4.19 when we ordered the autopsy confirmed families (n=228) by the covariate of VR22 LOD score, again suggesting independent effects of these two loci.

Taken together, these results indicate that the chromosome 12 locus acts independently of ApoE and VR22 to increase the risk of late-onset familial AD in a subset of families. These data suggest that the region between D12S1042 and D12S368 (~16 cM) should be the subject of further detailed genomic efforts for the disease.

A LOCUS ON CHROMOSOME 9p PREDISPOSES TO ACUTE ANTERIOR UVEITIS, A SPECIFIC DISEASE MANIFESTATION IN ANKYLOSING SPONDYLITIS. *J. Luo¹, T.M. Martin², J. Luo¹, T.M. Doyle², J.E. Coffman², F.I. Mackensen³, J.D. Reveille⁴, J.T. Rosenbaum².* 1) Dept Environmental Health, Univ Cincinnati, Cincinnati, OH; 2) Casey Eye Institute, Oregon Health & Science University, Portland, OR; 3) Ophthalmology, University of Heidelberg, Heidelberg, Germany; 4) Rheumatology, University of Texas-Houston Health Science Center, Houston, TX.

A genome-wide scan was conducted to search for loci associated with acute anterior uveitis. Families with 2 or more members who have acute anterior uveitis and/or ankylosing spondylitis (AS) were recruited. Evidence for linkage was determined by nonparametric multipoint linkage analysis using Gene Hunter Plus. The transmission disequilibrium test was also performed. The genome-wide scan for acute anterior uveitis was compared to one for AS performed by the North American Spondylitis Consortium (NASC). Out of 978 individuals genotyped, 244 were affected with acute anterior uveitis. The data presented represent 76 affected sibling pairs for acute anterior uveitis. Of these, 6 sib pairs denied any symptoms of AS, 12 uveitis sib pairs were discordant for AS and the remaining 58 uveitis sib pairs were concordant for AS, either by self-report or by radiographic disease determination. A region at chromosome 9p showed a striking association with uveitis but not AS. The maximum LOD score at this region was 3.69 for uveitis, while the LOD score for the NASC AS cohort was not significant (<1.0). A distinct AS cohort described by Laval, et al. showed suggestive linkage at this 9p locus. Additional regions with significant linkage include the MHC locus on chromosome 6, and a region at chromosome 1q. However, these regions also showed significant linkage with AS. Transmission disequilibrium analyses supported the genome-wide scan linkage findings, including the chromosome 9p uveitis linkage. A genetic region for acute anterior uveitis has been identified by genome-wide scan. These data reveal a region on chromosome 9p as a predisposing factor in uveitis. Comparison to a companion AS scan indicates that this region may be unique to the uveitis phenotype.

Polymorphism and linkage disequilibrium patterns at the serotonin transporter gene locus (SLC6A4). H. Mansour, M.E. Talkowski, J. Wood, K.V. Chowdari, M. Bamne, A. Fagiolini, E. Frank, M.E. Thase, D.J. Kupfer, V.L. Nimgaonkar. Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA, USA.

Polymorphisms of the serotonin transporter, SLC6A4 (17q11.1-q12), have been associated with mood disorders, as well as therapeutic response to some antidepressants. Yet, association studies have been inconsistent. Most studies have only investigated an insertion/deletion polymorphism (5HTTLPR) in the promoter region. We reasoned that other polymorphisms may contribute to the associations and the inconsistencies may be due to variations in linkage disequilibrium (LD) patterns. Therefore, we conducted LD analyses at SLC6A4.

We analyzed 5HTTLPR, as well as 11 additional polymorphisms across the SLC6A4 locus. Variation in 5HTTLPR was analyzed by resequencing, as well as a PCR based assay (Heils et al, 1996). Genotype assays for the SNPs were based on multiplex PCR followed by single base extension analysis (SnaPshot assays, ABI Inc). Our sample consists of 135 US Caucasian nuclear families having a proband with Bipolar I Disorder (BD1), available parents, and 73 patients with Major Depressive Disorder (MDD, DSM IV criteria). Cord blood samples from local Caucasian live births serve as unrelated, unselected controls (n = 182). We have evaluated pair-wise (LD) patterns separately between the SNPs among BD1 patients, their parents and the controls using r^2 and D value. Using D value, significant LD was observed among 6 SNPs, (rs140701, rs2020942, rs6354, rs2020939, rs2020937, rs2066713). Significant LD was also noted separately between 3 additional SNPs, (rs2020935, rs2020934 and rs2020933). Our analysis indicates 5HTTLPR is in significant LD with only one SNP (rs2020934). Further, another SNP flanking 5HTTLPR that is localized to the repeat motif (rs25532) is not in LD with the other SNPs. Our analyses in over 200 individuals (cases, parents and controls) suggest that rs25532 is monomorphic among all individuals homozygous for the 5HTTLPR insertion. Further association analyses with BD1 and MDD are currently ongoing. These analyses may help resolve some of the persistent controversies involving the etiological role of SLC6A4 in mood disorders.

Association of ACE polymorphisms and Alzheimer disease. *E.R. Martin¹, P. Bronson¹, R.H. Chung², P-T. Xu¹, J.L. Haines³, J. Gilbert¹, M.A. Pericak-Vance¹.* 1) Duke University Medical Center, Durham, NC; 2) North Carolina State University, Raleigh, NC; 3) Vanderbilt University Medical Center, Nashville, TN.

Several studies have evaluated the gene for angiotensin converting enzyme (ACE) on chromosome 17 for association with Alzheimer disease (AD). Though association studies have been conflicting, a recent meta-analysis concluded that the ACE gene is likely to play a role in AD risk (Elkins et al 2004). Despite this conclusion, the specific mechanism underlying this association has yet to be identified and most studies have looked at a single insertion/deletion polymorphism in intron 16 of the ACE gene. To further examine the effect of ACE variants in AD, we examined seven polymorphisms in the gene. Markers were genotyped in a large AD family dataset (324 families with 1,073 phenotypically discordant sibling pairs) and an independent case-control data set (281 cases and 677 controls). Many of these families, singleton cases and controls have not been tested for association with ACE polymorphisms previously. We found significant association with four ACE SNPs (p-values between 0.007 and 0.04) in the family dataset using the pedigree disequilibrium test. Two SNPs also showed association in the independent case-control sample (p-values=0.028 and 0.030), and this association remained when we adjusted for age, sex and APOE-4 status. The replication of significant association in independent family and case-control samples provides strong support for the role of ACE in Alzheimer disease. Despite our findings of association with several markers, the strong linkage disequilibrium between markers makes it difficult to distinguish which polymorphisms may be contributing to risk. Further study is warranted to understand the role of specific polymorphisms and the biological mechanism affecting AD risk.

Type I punctate palmoplantar keratoderma: refinement of the locus on chromosome 15 and evidence for genetic heterogeneity. A. Martinez-Mir¹, A. Zlotogorski⁶, D. Londono⁴, D. Gordon⁴, A. Grunn³, E. Uribe⁵, L. Horev⁶, I.M. Ruiz⁵, N.O. Davalos⁵, O. Alayan⁶, J. Liu³, T.C. Gilliam³, J. Miljkovic⁸, A. Stanimirovic⁹, J.C. Salas-Alanis⁷, A.M. Christiano^{1, 2}. 1) Dept Dermatology, Columbia University, New York; 2) Dept Genetics & Development, Columbia University, New York; 3) Columbia Genome Center, Columbia University, New York; 4) Lab of Statistical Genetics, Rockefeller University, New York; 5) Hosp Gral Occidente Zapopan, Mexico; 6) Dept Dermatology, Hadassah-Hebrew University Medical Center, Jerusalem; 7) Serv Medico Univ Autonoma Nuevo Leon, Mexico; 8) Maribor Teaching Hospital, Slovenia; 9) Medical School Zagreb University, Croatia.

Palmoplantar keratodermas (PPK) are a heterogeneous group of disorders of keratinization for which only a few molecular defects have been described. We report the identification and fine-mapping of a locus for type I punctate PPK. A genomewide scan was performed on an autosomal dominant pedigree and linkage to chromosome 15 was established. With the addition of two new PPK families of diverse geographical origin, we confirmed the mapping of the locus to a 9.98-cM interval, flanked by D15S534 and D15S818 (LOD score of 4.93, at D15S988). In order to refine the location of the PPK locus, we genotyped all available markers within the region and narrowed the locus to an interval of 8.89 cM, corresponding to a region of 3.7 Mb. We have recently identified 6 new PPK families from Slovenia. To evaluate the role of the PPK locus in this group of families, markers covering the interval on chromosome 15 were genotyped in those pedigrees with two or more affected individuals. The PPK locus on chromosome 15 cosegregated with the disease phenotype in all pedigrees tested. Very recently, Zhang et al have reported the identification of a second locus for punctate PPK on chromosome 8 in a Chinese pedigree. Genotyping of markers in this region failed to show cosegregation with the disease in our pedigrees, suggesting genetic heterogeneity. The results reported here will help in the identification of a new gene involved in skin integrity that could also contribute to the understanding of the multiple organ involvement in syndromic forms of PPK.

Identification of a locus for exudative age-related macular degeneration on chromosome 16p. *V.P.M. McConnell¹, G. Silvestri², A.E. Hughes¹*. 1) Department of Medical Genetics, Queen's University, Belfast, United Kingdom; 2) Ophthalmology and Vision Science, Queen's University, Belfast, United Kingdom.

Age-related macular degeneration (AMD) is the leading cause of blindness in elderly Caucasians. The exudative form, which is characterized clinically by choroidal neovascularization and fibrous scarring of the macula, is responsible for 80% of AMD-related blindness. Genetic predisposition in AMD is now well established, but remains largely undetermined. The aim of our study was to identify a locus for exudative AMD in a large family with several affected members, and to assess the importance of this locus in the Northern Irish population. Thirty members of the family were examined over a ten-year period and phenotyped using the Rotterdam AMD grading system for stereo-digital fundus photographs. A genome-wide screen was undertaken using 400 highly polymorphic microsatellite markers with linkage analysis restricted to affected individuals. The disease segregated as an autosomal dominant trait and mapped to 16p12-p13. Further refinement using additional microsatellite markers and haplotype analysis suggested that the disease gene was located between 3 and 19Mb. The maximum multipoint Lod score of 3.2 was obtained near D16S3103 (17.6Mb). Two disease haplotypes segregated in different branches of the family. An association study was performed using 200 unrelated exudative AMD cases and a similar number of controls, using clustered markers close to candidate genes. The most significant association found to date was near EMP2 ($p=0.01$), however, no mutation was detected in this gene within the large family. This is the first disease locus to be identified for exudative AMD. The combination of familial linkage and positive association data implies that the causative gene on chromosome 16p may play an important role in AMD.

Autosomal dominant Nocturnal enuresis: A study of an Indian Family. *R. Meda*¹, *J.V. Solanki*², *U. Ratnamala*¹, *U. Radhakrishna*¹. 1) Molecular Genetics Laboratory, Green Cross Blood Bank & Gen, Ahmedabad, India; 2) Department of Animal Genetics & Breeding, Veterinary College, Gujarat Agriculture University, Anand, India.

Nocturnal enuresis, or nightly bedwetting, is most common in children and affects approximately 15-20% of school age children between the ages of 4 and 16 and it continues to present in 2-3% adults. It is more common among boys than girls and about 5 to 7 million children wet the bed. Several factors cause bed-wetting; some of these include hormonal factors, Urinary tract infections, abnormalities in the spinal cord, and bladder size. In addition, Genetic factors also contribute to the pathogenesis. Several families with autosomal dominant and autosomal recessive modes of inheritance have been reported. The genes responsible for autosomal dominant (ENUR1 (OMIM 600631) and ENUR2 (OMIM 600808)) have been mapped to chromosome 13q13-q14.3 (Nature Genet. 10: 354-356, 1995) and 12q13-q21 (J. Med. Genet. 34: 360-365, 1997) respectively, but no mutation-causing gene has yet been identified. We have studied a large five-generation Indian pedigree for bedwetting in which the anomaly segregates as an autosomal dominant trait. The pedigree consists of 27 individuals with 11 affected (eight males and three females) and the age distribution of these affected is from 5-48 years. There are no other associated anomalies present in this family. We have genotyped this family for all polymorphic microsatellite markers flank the disease loci at chromosome 13q13-q14.3 and 12q13-q21. These markers were selected since they were used for the original linkage analysis for autosomal dominant ENUR1 and ENUR2. Analyzing 15 individuals, all markers yielded significant negative (-2.0) at = 0. Thus ENUR1 and ENUR2 can be excluded as the candidate region responsible for nocturnal enuresis in this family. We are planning to perform genome-wide linkage analysis in this family to identify the responsible locus. Email: u_c_rao@hotmail.com.

A novel locus for pure recessive Hereditary Spastic Paraplegia maps to chromosome 10q22.1-10q24.1. I.A.

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The Hereditary Spastic Paraplegias (HSP) are a group of clinically and genetically heterogeneous disorders characterized by progressive lower limb spasticity. In this study we present a single generation French Canadian family with autosomal recessive pure HSP. We analyzed the family for linkage to the known recessive loci at the start of the study (*SPG5*, 7, 11) and no evidence for linkage was found. We then proceeded to study the dominant HSP loci (*SPG3*, 4, 6, 8, 9, 10) in the family. A significant LOD score of 3.04 was obtained for marker D10S1755 (*SPG9*) with two point parametric linkage analysis. A maximum LOD score of 4.49 at theta 0 was found for more telomeric markers: D10S1786 and D10S1765. Haplotype analysis identified a compound heterozygote haplotype and two recombinants, which defined a critical interval of approximately 26Mb flanked by D10S606 and D10S1758. This novel recessive locus *SPG27* partially overlaps (6.1Mb) with the existing *SPG9* locus. We have either mapped a novel locus for pure recessive HSP to chromosome 10q22.1-10q24.1 or we have identified the first case of allelic disorders with different mode of inheritance in HSP. If the disorders are indeed allelic, our results have reduced the *SPG9* interval by 3Mb.

Linkage Analysis at the IFNGR1 Locus in Visceral Leishmaniasis. *L.M. Olson¹, N. Schnetz-Boutaud¹, R.R. Rodriques², R. Dietze², C.E. Carter³, C. Li¹, J.L. Haines¹.* 1) Center for Human Genetics Research, Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, Tennessee; 2) Nucleo de Doencas Infecciosas, Universidade Federal do Espirito Santo-UFES, Brazil; 3) Department of Biological Sciences, Vanderbilt University, Nashville, Tennessee.

Leishmaniasis is a parasitic infection transmitted by the bite of a sand fly. The two major forms of the disease are cutaneous and visceral leishmaniasis (VL). Cutaneous leishmaniasis is an infection of the skin that produces multiple ulcers or sores. Visceral leishmaniasis or Kala-azar is an infection of the internal organs and may produce fever, weight loss, anemia, and enlarged liver or spleen. VL is usually fatal if left untreated. We hypothesized that genetic variation underlies part of the variation in response to infection. Initial analysis focused on sixteen SNPs in and near six genes related to immune response. The six genes included IL10 on chromosome 1, IL4 on chromosome 5, IFNGR1 and TNF on chromosome 6, IFNG on chromosome 12, and IL12RB1 on chromosome 19. In preliminary linkage analysis, the data set consisted of seven multiplex VL families which showed a non-parametric multipoint LOD score, HLOD, of 1.66 at the hCV7578627 SNP in the IFNGR1 gene. After such encouraging linkage results, eleven additional trio families were included in the association analysis. The pedigree disequilibrium test (PDT), Geno-PDT and Tsp were used to assess association and linkage. The most significant result, though marginal with a p-value = 0.08, was the association of maternal transmissions for the Tsp or associated transmission/disequilibrium test (TDT) for SNP hCV7578627. Severity in leishmaniasis may be accounted for by analyzing additional SNPs to further explore the linkage and association in IFNGR1.

Linkage and association analysis of chromosomal 12q13 region in Italian atopic asthmatic families. *C. Patuzzo*¹, *C. Migliaccio*², *G. Malerba*¹, *R. Galavotti*¹, *S. Venanzi*³, *E. Trabetti*¹, *P.F. Pignatti*¹. 1) Sect Biol & Genetics, Mother-Child, Biol & Genetics, Verona, Italy; 2) International Institute of Genetics and Biophysics, CNR, Naples, Italy; 3) Department of Cellular Biology and Neurosciences, ISS, Rome, Italy.

Several reports have identified the chromosomal region 12q as related to asthma and atopic phenotypes in different populations. Our previous linkage analysis of chromosome 12 markers with allergic asthma phenotypes in 116 families from North East Italy (Malerba et al. *AJRCCM* 2000; 162:1587) indicated suggestive linkage with marker D12S390 (51.1 Mb from pter, Ensembl map v22). The highest multipoint lod score estimation was for the clinical asthma phenotype (mlod = 2.81, p=0.003). No linkage was thereafter observed for any phenotype and a CA repeat in the first intron of the Interferon γ (IFNG) gene (66.8 Mb from pter) or three intragenic microsatellites in the neuronal Nitric Oxide Synthase 1 (NOS1) gene (116.1 Mb from pter). The present study is aimed to improve the definition of the region near the D12S390 marker. For this purpose the sample of 45 families with at least one asthmatic sib pair was used for linkage disequilibrium mapping and a nested association-based study. A set of five microsatellite markers between D12S368 and D12S1644, covering a distance of 5 Mb, was used as well as SNPs in three different genes: keratin 4-type II cytoskeletal 4 or cytokeratin 4 (KRT4), eukaryotic translation initiation factor 4B (EIF4B) and signal transducer and activator of transcription 6 (STAT6). TDT analysis was performed on estimated haplotypes. The TRANSMIT programme was used to evaluate TDT and the significance was assessed with Bootstrap procedure. We obtained a TDT X2 value of 4.07 with P=0.0053 for the KRT4 gene and the clinical asthma phenotype. Multipoint linkage analysis (GENEHUNTER 2.1 computer programme) showed positive but non significant results. Further studies to verify the association data on the KRT4 gene will be necessary.

Linkage of the Metabolic Syndrome to a Genetic Location on Chromosome 7q in Mexican Americans. S.

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The metabolic syndrome (MS) consists of a cluster of related disorders including central obesity, dyslipidemia, insulin resistance and hyperinsulinemia, hyperglycemia and hypertension. Several epidemiological studies have shown that MS is a predictor of both type 2 diabetes (T2D) and coronary heart disease (CHD). Recently, the National Cholesterol Education Program / Adult Treatment Panel III (NCEP/ATPIII) proposed that MS requires the presence of at least 3 of the following 5 risk factors: increased waist circumference (102 cm in men or 88 cm in women), hypertriglyceridemia (150 mg/dl), low HDL cholesterol (40 mg/dl in men or 50 mg/dl in women), hypertension (130/85 mm Hg), and high fasting glucose (110 mg/dl). Given the epidemic of MS, the NCEP/ATPIII definition for MS has been used widely in both clinical practice and epidemiology. However, knowledge of its genetic basis is extremely limited. We therefore performed a genome scan of the MS phenotype defined in accordance with the NCEP/ATPIII criteria, using variance components linkage analysis, to identify major susceptibility loci for the MS with the program SOLAR. We analyzed phenotypic data from 741 participants distributed across 39 low-income Mexican American families in the San Antonio Gallbladder Study (SAFGS), for whom a 10-15 cM map is available. The prevalence of MS in these families was 44%. Perhaps for the first time, after accounting for the covariate effects of age, we found strong evidence (lod score = 3.6, P = 0.00002) for a major locus on chromosome 7q between the markers D7S2212 and D7S821, which influences MS. Suggestive evidence for linkage was found on regions of chromosomes 8q near the marker D8S1136 (lod score = 2.6) and 11p near the marker D11S1999 (lod score = 2.3). A positional candidate gene in our chromosomal region of interest is *CD36* (fatty acid translocase). In conclusion, we found strong evidence for linkage of MS in the Mexican American population to a region on chromosome 7q.

First genomic screen of Neural Tube Defects reveals regions of interest on chromosomes 7 and 10. E.

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Birth defects are the leading cause of death in babies under 1 year of age. Neural tube defects (NTD) with a frequency of 1/1000 in American Caucasians are the second most common birth defect. Periconceptional maternal folate supplementation has been shown to reduce NTD risk by 50-70%; however, studies in humans of folate-related genes and other developmental genes have failed to identify a major gene. The etiology of NTDs remains unknown. A traditional linkage approach to identify regions of interest is complicated in NTDs due to a paucity of families with two or more affected individuals available for sampling. We present findings from a genome scan of 44 multiplex pedigrees. Multipoint parametric and nonparametric analyses were performed using Allegro. To account for clinical heterogeneity we used a broad phenotype definition to include all NTDs and then allowed only families with two or more persons with lumbosacral myelomeningocele. Initial results revealed several regions of interest, including chromosomes 7 and 10 both with max parametric lod scores > 2.0 . Chromosome 7 produced the highest lod score (MLOD=2.45 at D7S3056) and showed consistent linkage evidence across all analyses. Further analyses demonstrated that results on chromosome 7 were being primarily driven by a single large pedigree, family 8776 that by itself generated a lod score of 2.40. When this family was removed from analysis, chromosome 10 was the most interesting region with a peak lod score of 2.12 at D10S1731. The availability of newly emerging technology via the Affymetrix Genechip Mapping 10K SNP Array allowed us to perform an independent screen of family 8776 by itself using a dense map. A comparison of linkage results from both screens is presented. Since multiplex NTD families are difficult to find, this screen is likely to be the only one performed with little chance for replication. Thus the synthesis of these results represents a valuable and novel resource for the identification of susceptibility genes for NTD.

Combined analysis of genome scans from five twin cohorts to locate quantitative trait loci for body mass index and stature in the GenomEUtwin project. *S. Sammalisto¹, T. Hiekkalinna¹, N. Martin², J. Harris³, D. Boomsma⁴, K. Christensen⁵, K. Ohm Kyvik⁵, N. Pedersen⁶, T. Andrew⁷, T. Spector⁷, E. Widen⁸, A. Palotie⁸, L. Peltonen¹, M. Perola¹, GenomEUtwin.* 1) Dept Molecular Medicine, Natl Public Health Inst, Helsinki, Finland; 2) Queensland Institute of Medical Research, Brisbane, Australia; 3) Norwegian Institute of Public Health, Oslo, Norway; 4) Free University, Amsterdam, Netherlands; 5) University of Southern Denmark, Odense, Denmark; 6) Karolinska Institutet, Stockholm, Sweden; 7) St Thomas Hospital, London, United Kingdom; 8) University of Helsinki, Finnish Genome Center, Finland.

European twin cohorts provide a unique competitive advantage for investigations of the role of genetics and environment or life style in the aetiology of common traits. The EU-funded GenomEUtwin (www.genomeutwin.org) consortium consists of eight twin cohorts (Australian, Danish, Dutch, Finnish, Italian, Norwegian, UK and Swedish). As a proof-of-principle study for joint analyses of these large cohorts, we performed QTL analysis of body-mass index (BMI) and stature (body height) using genotypic data from six genome-wide scans performed on the GenomEUtwin participating countries. The study material consisted of 5723 individuals: Australia (n=1323), Finland (n=879), Netherlands (n=876), Sweden (n=102) and United Kingdom (n=2228). The genetic marker maps were integrated using physical location information and interpolation the genetic distances from the DeCode genetic map, using its markers as an anchoring set (www.bioinfo.helsinki.fi/cartographer). The raw marker data was pooled by a program developed by us, MERGESCAN, which uses the information from Cartographer to facilitate joint analysis for different datasets for combined genome-wide analyses. We used the program Merlin for variance components analysis with age, sex and cohort as covariates. The covariate adjusted heritability of BMI was found to be 64% and of stature 89% in the pooled data set. We found evidence for a QTL on chromosomes 6q, 8q and 10q determining human stature. We plan to integrate more data in these analyses from GenomEUtwin participants in near future.

A MHC-chromosome 2 interaction in JRA affected sibpairs. *M.L. Sudman¹, L. Guyer², M. Ryan², M.A. Keddache¹, W.M. Brown³, C.D. Langefeld³, S.S. Rich³, C. Valis³, W.C. Nichols¹, M.B. Moroldo², S.D. Thompson², D.N. Glass².* 1) Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 2) Division of Rheumatology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; 3) Wake Forest University School of Medicine, Winston-Salem, NC.

Juvenile Rheumatoid Arthritis (JRA) is a complex genetic disorder and is the most common chronic rheumatic inflammatory condition in childhood, with an estimated 50,000 children diagnosed in the US. The disease is phenotypically and genetically heterogeneous and distinct from adult onset RA. A genome scan (386 microsatellite markers) was done on 121 families containing 247 affected children (NIAMS supported JRA Affected Sibpair [ASP] Registry). In addition, individuals were typed for HLA-DR. Pedigree disequilibrium tests at the DRB1 locus revealed excess transmission of the DR8 haplotype to offspring with pauciarticular JRA (haplotype-specific $P = 2.0 \times 10^{-6}$). Other haplotypes, including HLA-DR11, 13 and 4, are well documented to be associated with susceptibility (or resistance) to one or more subtypes of JRA. To evaluate potential interactions between HLA-DR and other genomic regions, nonparametric linkage analysis was performed on 32 pedigrees with offspring concordant for the presence of at least one DR8 allele. The strongest evidence for linkage in these pedigrees was near D2S168 at 35 cM (2p25, LOD=6.00). Fine mapping with 13 additional microsatellites produces a LOD=6.09 and reduced the LOD-1 support interval to 7 cM (31.6-38.6 cM). Suggestive evidence for linkage in these 32 pedigrees was observed at D14S292 at 131.5 cM (14q32, LOD=1.84). Repeating these analyses on the pedigrees with at least one offspring having HLA-DR4 yielded a LOD=2.48 at 3p27, and on pedigrees concordant for the presence of either DR8, DR11 or DR13 yielded a LOD=2.34 at 17q25. Linkage disequilibrium analyses based on a dense SNP map of the 2p25 region are underway.

Environmental Covariates Yield Major Gains in Power in Linkage Analysis. *T.N. Turley-Stoulig¹, A.J.M. Sorant², J.E. Bailey-Wilson², D.M. Mandal¹.* 1) Dept. of Genetics, LSU Health Sciences Center, New Orleans, LA; 2) NHGRI/NIH, Baltimore, MD.

Inclusion of covariates in linkage analysis of complex traits may increase the power to detect linkage. To identify situations in qualitative trait linkage analysis with large power gain, this study evaluates the effect of inclusion of a covariate on power and type I error for qualitative trait linkage analysis under various inheritance models.

A binary trait with penetrance determined by a dominant biallelic locus and modified by a quantitative covariate was simulated with G.A.S.P., using various degrees of penetrance, disease allele frequency and covariate effect. For each model, segregation analysis was performed (REGDHUNT) on a large singly ascertained sample to provide a trait model for use in the model-based analysis. Then the trait and linked ($\alpha=0.01, 0.05$) and unlinked markers were generated for 10,000 samples of 300 nuclear families with 4 offspring. Each linkage sample was analyzed with model-based (LODLINK, S.A.G.E. 3.1) and model-free (revised Haseman-Elston regression SIBPAL, S.A.G.E. 4.5, using the mean-corrected cross-product as dependent variable) methods, with and without the covariate.

Under examined inheritance models, both LODLINK and SIBPAL provided more powerful linkage analysis and conserved type I error with covariate inclusion. Under models with moderate to strong genetic effect and moderate covariate effect, power to detect linkage with LODLINK was increased as much as 6-fold when the covariate was included in analysis.

Identification of two candidate loci for intracranial aneurysms in the French Canadian population. *D.J. Verlaan¹, M.P. Dubé¹, J. St-Onge¹, N. Satgé¹, M.C. Wallace², G.A. Rouleau¹*. 1) Centre for Research in Neurosciences, McGill University, Montreal, Canada; 2) Division of Neurosurgery Toronto Western Hospital, University of Toronto, Toronto, Canada.

Introduction: Intracranial Aneurysms (ICA) are a common and serious problem of uncertain etiology. ICA rupture is responsible for approximately 10% of all strokes. It is a serious condition because the first manifestation is almost always rupture, leading to death in 50% of cases and significant morbidity in an additional 30%.

Aim: The purpose of our study is to map a gene (or genes) that predisposes to intracranial aneurysms (ICA) in the French Canadian population.

Methods: An 8cM genome-wide scan was performed on a large French Canadian family affected with intracranial aneurysms (affected cases only). All individuals were successfully genotyped at 531 markers with a success rate of 95%. Haplotypes were reconstructed by using the Maxprob functions and multipoint linkage analysis was performed using the parametric and non-parametric functions of the Genehunter v.2.1 program. For the analysis, an autosomal dominant model with a disease allele frequency of 0.5% and a phenocopy rate of 5% was used. A high penetrance of 95% was used because an affected-only analysis was performed.

Results: Ten different loci were found to be slightly positive (LOD1). Further genotyping and analysis with another affected individual reduced the number of positive loci to 2. Multipoint analyses of the two loci show that they have a LOD score close or equal to 3. These loci are found on chromosome 12q23-24 and 5p15-14.

Conclusion: Genotyping of additional French Canadian families will permit us to determine if both or only one of these loci harbor a susceptibility gene for intracranial aneurysm in the French Canadian population.

Sex-specific genetic architecture of whole blood serotonin levels. *L.A. Weiss*¹, *M. Abney*¹, *E.H. Cook*^{2,1}, *C. Ober*¹. 1) Dept Human Genetics, Univ Chicago, Chicago, IL; 2) Dept Psychiatry, Univ Chicago, Chicago, IL.

Recently, we identified integrin 3 (*ITGB3*) on chromosome 17q as a quantitative trait locus (QTL) for whole blood serotonin in the Hutterites (Weiss et al., in press, Eur J Hum Genet). Because we found serotonin level to be sexually dimorphic in this population, in the current study we evaluated the sex-specific genetic architecture of serotonin. Whole blood serotonin was measured by HPLC in 300 females and 267 males, who were genotyped for 1,123 autosomal markers. Tests developed for QTL mapping in large pedigrees include a linkage method (homozygosity-by-descent [HBD]) and an association method (allele-specific HBD [ASHBD]) and utilize the probability of HBD at a locus or allele, respectively, as a predictor of the trait value (Abney et al., 2002, Am J Hum Genet 70:920-34). Both sexes had high broad heritability ($H^2=0.99$), but females had a higher additive component ($h^2_{\text{male}}=0.27$, $h^2_{\text{female}}=0.63$), suggesting that serotonin level shows sex-specific architecture. Both sexes contributed to a linkage signal on chromosome 12q ($\text{LOD}_{\text{male}}=2.0$, $\text{LOD}_{\text{female}}=0.99$, $\text{LOD}_{\text{all}}=2.4$) and 16p ($\text{LOD}_{\text{male}}=2.0$, $\text{LOD}_{\text{female}}=0.74$, $\text{LOD}_{\text{all}}=2.4$). In contrast, the serotonin QTL on 17q at *ITGB3* showed linkage in males only ($\text{LOD}_{\text{male}}=2.7$, $\text{LOD}_{\text{female}}<0.1$, $\text{LOD}_{\text{all}}=1.9$), as did a region on 2q ($\text{LOD}_{\text{male}}=2.1$, $\text{LOD}_{\text{female}}<0.1$, $\text{LOD}_{\text{all}}=0.61$). On the other hand, a region on 6q was specific to females ($\text{LOD}_{\text{male}}<0.1$, $\text{LOD}_{\text{female}}=2.8$, $\text{LOD}_{\text{all}}=0.99$). The association test showed overall more results of suggestive significance in males than females: 7 markers in 4 regions (including *ITGB3* and the serotonin transporter gene) vs. 1 marker, respectively. This analysis is consistent with heritable sexual dimorphism in whole blood serotonin levels resulting from a combination of sex-specific and sex-independent loci. As a result, effects contributed by a subgroup of the population (one sex) were missed in the undivided sample. If sex-specific genetics is as general a phenomenon in humans as it is in model organisms, mapping of complex traits may be enhanced by modeling for different architecture in males and females.

LD-PPL: the Posterior Probability of Linkage (PPL) with Linkage Disequilibrium (LD). *X. Yang¹, L.M.*

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PPL as a Bayesian method for pedigree data has been developed for linkage analysis based on direct measurement of posterior probability of linkage in the past years. One limitation of the method has been that it assumes linkage equilibrium in the population from which the families are sampled. When a marker locus is closely linked to the disease susceptibility loci, it is highly likely that they are in linkage disequilibrium (LD). One would assume that stronger linkage signals would be detected by taking LD into consideration if there are reasons to believe that LD exists. We have developed a method for directly measuring the linkage evidence with linkage disequilibrium (LD-PPL), which involves recovering marginal posterior density by integration over nuisance parameters including LD coefficient, locus heterogeneity parameter and other trait model parameters. Simulation studies have showed increased LD-PPL compared to PPL with increased LD level in a variety of generating models with linkage. It is also observed that LD-PPL is consistently smaller than corresponding PPL when the marker and the disease are unlinked. We have applied this method to a previously published schizophrenia data set, where strong LD has been detected between the region of CAPON on 1q22 and schizophrenia. 15 SNPs within this region are investigated. Stronger linkage signals have been obtained using LD-PPL than PPL suggesting the LD-PPL method may be more powerful for gene identification even in complex disorders.

Linkage study of the candidate gene *SCN2A1* in familial mesial temporal lobe epilepsy (FMTLE). C.V. Maurer-Morelli¹, N.F. Santos¹, R.B. Marchesini¹, R. Secolin¹, E. Kobayashi², F. Cendes², I. Lopes-Cendes¹. 1) Medical Genetics, FCM/UNICAMP, Campinas, SP, Brazil; 2) Neurology, FCM/UNICAMP, Campinas, SP, Brazil.

Purpose: Mutations of voltage-gated sodium channel genes are known to be responsible for some types of human epilepsy. In a recent report, a mutant *Scn2a* mice showed focal seizures activity in the hippocampus with extensive cell loss and gliosis within the CA1, CA2, CA3 areas and hilus. Recently, we described a large group of families segregating a distinct type of temporal epilepsy named FMTLE, with evidence of a strong genetic predisposition for the development of hippocampal atrophy. Complex segregation analysis provided evidence for the presence of a major gene segregating in this disorder. The objective of this study was to carry out linkage studies in FMTLE in order to investigate whether the human *SCN2A1* may play a role in the etiology of FMTLE.

Methods: We studied 29 unrelated families segregating FMTLE. Among these families we have identified two kindreds that are significantly informative for linkage analysis: F-10 and F-26. A total of 57 individuals, including 32 patients were analyzed in this study. We genotyped five polymorphic dinucleotide repeat markers flanking the *SCN2A1* region on chromosome 2q22-q23: D2S347, D2S129, D2S151, D2S2241 and D2S141. The screening was performed by PCR amplification. Two-point lod scores (Z) were calculated for each family separately using the LINKAGE package.

Results: Simulation analysis for the two kindreds (F-10 and F-26) indicates a Z_{max} of 8.9, 4.5 and 4.4 respectively. The two Brazilian families segregating FMTLE showed negative lod score for all markers genotyped, ranging from -1,22 to -3,40 at different recombination fractions.

Conclusion: Our results suggest that, although the hippocampal lesion in the mutant *Scn2a* transgenic mouse is similar to that found in FMTLE patients, the *SCN2A1* gene is not the major locus involved in the etiology of the disease in our patients.

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A novel Locus for Maternally Inherited Human Gingival Fibromatosis at Chromosome 11p15. *L. Hu¹, Y. Zhu¹, Z. Huo², W. Zhang³, Y. Zhang¹, Y. Xia¹, B. Li¹, X. Kong¹.* 1) Health Science Center, sibs., Shanghai, shanghai, China; 2) Ningxia Medical College, Yinchuan 750004, Peoples Republic of China; 3) Qingdao Haici Hospital, Qingdao 266033, Peoples Republic of China.

A novel Locus for Maternally Inherited Human Gingival Fibromatosis at Chromosome 11p15 ABSTRACT Human isolated gingival fibromatosis is an oral disorder characterized by a slowly progressive benign enlargement of gingival tissues. The most common form of hereditary gingival fibromatosis (HGF) is usually transmitted as an autosomal dominant trait. We reported here for the first time a newly identified maternally inherited gingival fibromatosis in two unrelated Chinese families was mapped to human chromosome 11p15 with a maximum two point lod score of 8.70 at D11S4046(= 0) for family 1 and of 6.02 at D11S1318 for family 2. A critical region from D11S1984 to D11S1338 was defined by haplotype analysis. A cluster of known maternally expressed genes is mapped within this interval and is considered as candidate genes. We have screened mutations in all known maternally expressed genes within this region for individuals of these two families, no mutation at coding sequence and intron-extron boundary of these genes was found. According to Knudson's hypothesis of "two hits", mutation or epigenetic mutation of an imprinted tumor suppressor may increase tumor susceptibility. This result provide valuable clues for the identification of a candidate tumor suppressor on human chromosome 11p15.5 and also provide first direct evidence that imprinting (or imprinted gene) mutation results in tumorigenesis.

Using population structure to map complex diseases. *B. Peng*¹, *W. Amos*², *F. Balloux*³, *M. Kimmel*¹. 1) Department of Statistics, Rice University, Houston, TX; 2) Department of Zoology, Univ. of Cambridge, Cambridge, UK; 3) Department of Genetics, Univ. of Cambridge, Cambridge, UK.

In population-based case-control association studies, population structure can lead to highly significant but spurious associations. The attempts to avoid this problem lead directly to the popularity of transmission disequilibrium tests and recently, some statistical methods that use genomic markers to control for population structure in population-based studies. The two main methods are Genomic Control which estimates the degree of overdispersion generated by population substructure, and Structured Association which uses marker data to estimate structure present in the data. However, these approaches have significant drawbacks when susceptibility factors are distributed heterogeneously in the sample. We propose an alternative approach. Instead of treating the sample as comprising a number of homogeneous units, we estimate the relatedness between pairs of individuals and then assume that more related individuals / families share more susceptibility factors in common than less related individuals / families. In this way we combine the measurement of structure with the estimation of degree of association between markers, flexibly and in a way that bypasses the need to estimate discrete sub-groups. Our method consists of three steps: 1) estimate family relatedness and combine this with data on marker location to construct the weighting system, 2) estimate locus-level inbreeding and association measures and obtain summary measurements using the relatedness-aware weighting matrix and 3) test the significance of each value using exhaustive randomizations. We have focused on the leprosy dataset of Siddiqui et al. (2001) to validate our method. Siddiqui and co-workers report a genetic linkage scan of the genomes of 224 families from South India, containing 245 independent affected sib-pair. In a two-stage genome screen using 396 microsatellite markers, they found significant linkage on chromosome 10p13. We use a dataset consisting of 394 individuals in 96 nuclear families. The results we obtain are very close to those obtained in the original study.

Linkage disequilibrium analysis of recombination hotspot at the upstream region of the α -globin gene. *J. Ohashi*¹, *I. Naka*¹, *J. Patarapotikul*², *H. Hananantachai*², *G. Brittenham*³, *A. Clark*⁴, *S. Looareesuwan*², *K. Tokunaga*¹. 1) Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; 2) Department of Microimmunology and Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; 3) Department of Pediatrics and Medicine, Columbia University, College of Physicians and Surgeons, New York, New York, USA; 4) Department of Molecular Biology and Genetics, Cornell University, Ithaca, New York, USA.

Recombination hotspot located upstream of the α -globin (HBB) gene has been intensively studied by pedigree analysis, sperm typing approach, and linkage disequilibrium (LD) analysis so far. Our previous study based on patterns of LD among 44 biallelic markers in Thai suggested a putative recombination hotspot spanning 2.0 kb in length at the upstream of the HBB gene (Ohashi et al., *AJHG* 74:1198-1208). The putative hotspot was found to include a binding site for the repressor protein BP1, which contains a tandem (AT)_x(T)_y repeat. In this study, to narrow the region of the recombination hotspot, we analyzed several polymorphic sites including (AT)_x(T)_y repeat in the putative recombination hotspot in Thai and Japanese populations. The following linkage disequilibrium and haplotype analyses suggested that this hotspot consisted of 683 bp and did not include the BP1 binding site. The localization of recombination hotspot would give valuable information for better understanding of the mechanism of recombination in the human genome.

Haplotype structure in the genes encoding thermosensitive receptors and experimental pain sensitivity in humans. *H. Kim, R.A. Dionne.* PNMB, NIDCR / NIH, Bethesda, MD.

Even though the individual gene effects may be small, interactions among the genes and environments may make a substantial contribution to the final manifestation of the pain sensitivity. The haplotype, a particular combination of alleles observed in populations, focuses on patterns of a few single nucleotide polymorphisms (SNPs) that define each haplotype and has contributed to the identification of genes for Mendelian disease and, recently, disorders that are common but complex in inheritance. Normal subjects (387 females and 260 males) from 4 major ethnic populations were evaluated for their heat and cold pain sensitivity and SNPs genotyped from genes encoding transient receptor potential A subtype 1 (*TRPA1*), transient receptor potential M subtype 8 (*TRPM8*), transient receptor potential V subtype1 (*TRPV1*) and -opioid receptor subtype 1 gene (*OPRD1*). The haploblocks based on solid spine LD method were generated by Haploview version 2.05 with SNPs whose minor allele frequency > 0.2, genotype success rate > 0.9. Departure from Hardy-Weinberg equilibrium was tested by a χ^2 test. Analysis of variance was performed to evaluate the association between haploblocks and heat and cold pain sensitivity. From these 4 genes region, there are 6 haploblocks in European Americans, 3 blocks in African Americans, 4 blocks in Hispanic, and 4 blocks in Asian Americans. We found differences in haplotypes among populations from screened genes except for the *TRPM8*, in which we could not find any haploblocks. We found significant associations between experimental pain sensitivity and haplotype blocks from *TRPA1* and *TRPV1* in this human cohort ($p < 0.05$). Our observations demonstrate that haplotypes of *TRPA1*, *TRPV1*, and *OPRD1* have different patterns in each ethnic population and those from *TRPA1* and *TRPV1* contribute to individual variations in experimental pain sensitivity.

Genome-wide SNP association studies for cardiovascular adverse reactions by calcineurin inhibitors in renal transplant recipients. *T. Mushiroda*¹, *Y. Ohnishi*¹, *S. Saito*¹, *T. Yamazaki*¹, *N. Kamatani*¹, *Y. Beck*², *Y. Nakamura*^{1,3}. 1) SNP Research Center, The Institute of Physical and Chemical Research (RIKEN), Tokyo, Japan; 2) Department of Surgery and Bioengineering, Advanced Clinical Research Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan; 3) Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan.

Cardiovascular events are relatively common and severe adverse outcomes caused by treatment with calcineurin inhibitors (CNIs). We conducted case-control association studies using gene-based 52,608 single nucleotide polymorphisms (SNPs) with 72 patients receiving cyclosporine or tacrolimus after renal transplantation to identify the susceptible genes to cardiovascular events. The overall incidence of cardiovascular events was 13.9% (ten patients) among 72 patients treated with either of the drugs. We observed associations with p-value of <0.001 at 19 SNP loci calculated by allele frequency model, at 14 SNP loci by dominant inheritance model, and at 8 SNP loci by recessive inheritance model. On the basis of information of the ten selected SNPs in order of significance of p-value, we attempted to establish a scoring system to predict the risk of cardiovascular toxicity of the treatment and found that the system well separated the patient groups according to absence or presence of ADR. Score distribution patterns in general Japanese populations are quite similar to those in the ADR-negative group, but quite different from those in the ADR-positive group, indicating that pharmacogenetic testing with the prediction scoring system may provide a good indicator for careful monitoring cardiotoxicity and for treating the adverse reaction at an early stage to avoid the fatal condition.

Autosomal Dominant Adult Detrusor Instability with Childhood Nocturnal Enuresis: Localization to a 12cM

Region on 13q14.11. *M.F. Buckley¹, T. Roscioli¹, G.L. Mullan¹, A. Morris², K. Moore², J.A. Donald³*. 1) Molecular & Cytogenetics Unit, Prince of Wales Hosp, Randwick, Australia; 2) Pelvic Floor Unit, St. George Hospital, Sydney, Australia; 3) Department of Biological Sciences, Macquarie University, Sydney, Australia.

Nocturnal Enuresis (NE) and Detrusor Instability (DI) is a common, complex genetic disorder of bladder control for which genetic loci have been reported on chromosomes 4, 8, 12, 13 and 22. No genes have been isolated for this condition. We report preliminary evidence for a novel locus in a further Caucasian family in which DI, NE and day wetting show an autosomal dominant pattern of inheritance. The twelve affected individuals in this family consist of ten adult females, one female child and one male adolescent. Two of the affected individuals were diagnosed with DI by urodynamic testing without clinical symptoms. A further five adult females had the clinical phenotype of DI, as did one female child with persistent day wetting. One individual was phenotypically normal but was an obligate carrier by pedigree and therefore likely to be non-penetrant. A partial genome scan was carried out using genetic markers at loci previously implicated in NE (D4S2960, D8S264, D12S80, D12S86, D13S263, D13S291, D22S343, D22S446). Preliminary LOD score estimates were calculated assuming an autosomal dominant pattern of inheritance, phase-known and with incomplete penetrance. The loci on chromosomes 4, 8, 12 and 22 did not segregate with the DI phenotype. A LOD score of 2.11 at D13S263 suggested linkage to 13q14.11. A higher resolution scan was therefore performed at 5cM intervals between the centromere and D13S263 on chromosome 13. Two alleles at D13S171 and D13S219 segregated with the phenotype, being present in all affected and obligate carrier individuals. The locus D13S263 that was previously implicated in NE lies telomeric to the region defined in this study. Lack of recombination between loci D13S171, D13S219 and the phenotype suggests linkage despite the modest LOD score for D13S171. Analysis of a further two informative individuals and reanalysis of the data using multipoint linkage is in progress.

A first-pass genome-wide scan for susceptibility to amyotrophic lateral sclerosis using 2,500 microsatellite markers. *A. Al-Chalabi¹, V.K. Hansen¹, C.L. Simpson¹, J. Knight², B. Neale², P.C. Sham², M.R. Turner¹, C.E. Shaw¹, P.N. Leigh¹, J.F. Powell³.* 1) Neurology, Institute of Psychiatry, London, United Kingdom; 2) Statistical Genetics, Institute of Psychiatry, London, United Kingdom; 3) Neuroscience, Institute of Psychiatry, London, United Kingdom.

ALS is a relentlessly progressive fatal neurodegenerative disorder of motor neurons. In about 2% of all cases, mutations are found in the superoxide dismutase 1 gene (SOD1). Numerous candidate gene screens have yielded inconsistent results, possibly because of phenotypic heterogeneity. We performed a genome-wide scan for association using microsatellite markers using a two-stage design in which a non-stringent first pass is followed by a stringent replication pass.

Individuals from a clinic-based population were selected on the basis of disease severity. Controls were from the same geographical region and matched for age and sex. DNA pools were made with the highest and lowest quintiles for age of onset, and the remainder split by clinical phenotype (limb or bulbar onset of symptoms). Microsatellites were selected to target gene-dense regions or candidate genes and typed on an ABI 3100 genotyper (Applied Biosystems). For the first pass, results were prioritised using metaregression of allele frequencies against phenotype weighted by the inverse of the measurement variance.

There were 300 cases and 300 controls with 60 in each of the age of onset pools, 123 in the limb onset and 57 in the bulbar onset pool. More than 3000 microsatellites were typed, for which results were obtained for 2210. The metaregression analysis was neither conservative nor anticonservative. There were 6 markers with $p < .01$. The most significant result was for marker D3s1298 with $p = .0001$.

This is the first genome-wide analysis of sporadic ALS. A replication study of the 6 positive markers in a second population is required.

A QTL on chromosome 11q determines decrease of mean arterial blood pressure over time despite weight gain in a single Mexican American family. *G. Cai¹, J.W. Kent, Jr.¹, R.A. Bastarrachea¹, S.A. Cole¹, L. Atwood², J. Blangero¹, J.W. MacCluer¹, A.G. Comuzzie¹.* 1) Genetics, SW Foundation for Biomed Res, San Antonio, TX; 2) School of Medicine, Boston Univ.

Although genome scans have been performed to search for quantitative trait loci (QTLs) influencing variations in blood pressure, none has examined rate of change in mean arterial blood pressure (MBP, mm Hg/year). We conducted a genome wide scan to localize QTLs for average changes of systolic, diastolic, and mean arterial blood pressures (rSBP, rDBP, rMBP) in 653 Mexican Americans using SOLAR. A significant linkage for rMBP was found near marker D11S4464 (LOD = 5.42). The same marker also was linked to rSBP and rDBP (LOD = 2.78 and 3.99, respectively). Interestingly, pedigree-specific LOD analysis revealed that a single family of 62 members contributed 3.92 to the total LOD score of 5.42. In contrast to the general population where blood pressures increase when people get older, this family presented a decreasing trend of SBP, DBP, and MBP, although they did get heavier and had larger waist circumferences. Also, six members of this family are diagnosed as hypertensive. Out of the 62 members in this family, 35 members use alcohol and 27 smoke. However, no genotype by drinking or by smoking interaction was detected. The trait presented as a bimodal distribution in this family after accounting for the effect of covariates of smoking, drinking, sex, age, age-squared, sex by age interaction, and yearly change in waist circumference, suggesting a single allele dominant pattern with incomplete penetrance. The QTL on chromosome 11q does not overlap regions previously identified for variations in blood pressure in the same data set. This implies that distinct genes contribute to the variation in rMBP. Given the magnitude of the signal, the fact that the linkage signal came from a few dozen people, the unique phenotype (decreasing blood pressures in spite of weight gain), and the bimodal distribution of the trait, which is classic for a single allele effect, it is highly likely that we will be able to find the causal gene(s) through future fine mapping and single nucleotide polymorphism analysis.

A genome-wide search for creatinine clearance QTLs in West Africans with Type 2 Diabetes. *G. Chen¹, A. Adeyemo^{1,2}, J. Zhou¹, T. Johnson³, A. Amoah⁴, J. Acheampong⁵, G. Okafor⁶, Y. Chen¹, G. Dunston¹, F. Collins⁷, C. Rotimi¹.* 1) Natl Human Genome Ctr, Howard Univ, Washington, DC; 2) University of Ibadan, College of Medicine, Ibadan, Nigeria; 3) University of Lagos, College of Medicine, Endocrine and metabolic Unit, Lagos, Nigeria; 4) University of Ghana Medical School Dept. of Medicine, Accra, Ghana; 5) University of Science and Technology Dept. of Medicine, Kumasi, Ghana; 6) University of Nigeria Teaching Hospital Dept. of Medicine, Enugu, Nigeria; 7) National Human Genome Research Institute, NIH, Bethesda, MD.

Chronic renal failure occurs with a frequency of 200 cases per 1 million persons and two-thirds of all cases are associated with diabetes and/or hypertension. Creatinine clearance is commonly used to estimate renal function in clinical practice and a decreased creatinine clearance is an early sign of CRF. We have investigated the genetic determinants of creatinine clearance by conducting a genome wide search for QTLs linked to creatinine clearance in 691 type 2 diabetes patients (321 sib pairs and 36 half-sib pairs) in West Africa. We defined the phenotype using two residual models for creatinine clearance: a basic model (that included fat mass, fat-free mass, pulse rate, pulse pressure and glucose as covariates) and a full model (that included all the variables in the basic model as well as C-peptide levels). Suggestive linkage evidence was found in three genomic regions: 7p13 (peak LOD score = 2.20, $p = 0.0007$ @55cM), 10q22 (peak LOD score = 2.0, $p = 0.0012$ @93cM) and 2q14 (peak LOD Score = 1.95, $p = 0.0014$ @133cM) using the full model. Linkage peaks were found in the same regions using the basic model but these did not reach suggestive linkage evidence level. Candidate genes in or around the linkage regions include: GCK, OGDH, HK1 (involved in glucose metabolism) and GPR17 (transduce cellular signals). In summary, there are QTLs showing linkage to creatinine clearance in this study and the inclusion of C-peptide in the genetic model improved the ability to detect these loci.

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Linkage study of panic disorder: genome screen and fine mapping. A..J. Fyer¹, S.P. Hamilton³, G.A. Heiman¹, V. Haighighi^{1,2}, M. Durner¹, A. deLeon¹, O.V. Evgrafov¹, M. Bautista³, R. Costa¹, P.a. Tabares¹, P. Adams¹, T.C. Gilliam^{1,2}, D.F. Klein¹, S.E. Hodge^{1,4}, J.A. Knowles¹, M.M. Weissman¹. 1) Dept Psychiatry, Columbia University/NYSPI, NY, NY; 2) Dept Genetics and Development, Columbia University, NY, NY; 3) Dept of Psychiatry, UCSF, San Francisco, CA; 4) Dept of Biostatistics, Columbia University, NY, NY.

Panic disorder is a common illness characterized by recurrent, unpredictable episodes of intense anxiety. Significant evidence indicates a genetic contribution to etiology with heritability estimated at 35-49% but no specific mode of transmission. We previously reported a genome-wide screen (average spacing 9cM) in 120 multiplex panic disorder pedigrees. Here we present further work in this sample. Genome scan data have been re-analyzed using additionally available genotypings and clarification of family structure. Analyses were carried out under two modes of inheritance (dominant, recessive) using three pre-designated diagnostic thresholds for panic disorder (Broad, Intermediate, Narrow). Two-point and multipoint lod scores (under assumption of homogeneity and heterogeneity), as well as NPL scores were calculated for each phenotype-model combination. Consistent with previous results, three regions (chromosomes 2, 9, 15) received convergent support for suggestive linkage from at least two of the three analytic methods (i.e. two point, multipoint, NPL). Fine mapping (2-4cM) data from each of these regions are being analyzed and will be presented. In addition, as panic disorder is twice as common in females as males, we will also present analyses of the genome-wide data allowing for independent male and female recombination fractions.

Genetics of recurrent early-onset major depression: Genome scan linkage analysis of the full GenRED study sample. *P. Holmans*¹, *M.M. Weissman*², *G.S. Zubenko*³, *J.R. DePaulo, Jr.*⁴, *R.R. Crowe*⁵, *W.A. Scheftner*⁶, *P. Adams*², *J.A. Knowles*², *D. March*², *W.N. Zubenko*³, *M.G. McInnis*⁴, *D. MacKinnon*⁴, *K. Murphy-Eberenz*⁷, *M. Gladis*⁷, *S. Boutelle*⁵, *D.H. Marta*⁶, *J. Thomas*⁴, *E. Miller*⁴, *D.F. Levinson*⁷. 1) Dept Psychological Medicine, UWCM, Cardiff, United Kingdom; 2) Dept Psychiatry, Columbia Univ, and NY State Psychiatric Institute, NY, NY; 3) Dept Psychiatry, Univ of Pittsburgh, Pittsburgh, PA; 4) Dept Psychiatry, Johns Hopkins Univ, Baltimore, MD; 5) Dept Psychiatry and MHCRC, Univ of Iowa, Iowa City, IA; 6) Dept Psychiatry, Rush Univ Medical Center, Chicago, IL; 7) Dept Psychiatry, Univ of Pennsylvania, Philadelphia, PA.

The GenRED (Genetics of Recurrent Early-Onset Depression) project has collected 680 families containing 927 independent affected sibling pairs plus additional non-sibling affected relatives. DNA specimens and diagnostic data will be made available by NIMH. The primary allele-sharing analysis (ALLEGRO) of all 685 informative affected pairs in the first 297 families demonstrated genome-wide significant linkage (Z-likelihood ratio=4.14, $p=0.023$, 103.2 cM) on chromosome 15q (AJHG 74:1154-1167, 2004). In the full sample (1,508 pairs), 15q was the most positive region, but at the suggestive level (Zlr=2.98 at 105.4 cM); the second part of the sample (Zlr=0.31) did not add support for linkage. Zlr>2.0 was observed on chromosomes 1 (211.16 cM), 5 (190.77 cM) and 8 (37.84 cM). In a secondary logistic regression analysis with sex as a covariate (M-M, M-F, F-F pairs), linkage was observed on chromosome 17q (28.0 cM, lod=1.11 without and 4.37 with covariates, chromosome-wide $p=0.0012$, reaching genome-wide significance), with excess sharing in M-M (0.58) and M-F (0.56) pairs. Modest evidence for sex effects was observed on chromosomes 6 and 8. On chromosome 12, where Abkevitch et al. (AJHG 73:1271-1281, 2003) reported significant linkage in males (110 cM), excess sharing was observed at 100.4 cM in M-M pairs in both parts of the scan (0.61, 0.57) and overall (0.59), but results did not reach significance here. These results suggest that chromosomes 15q and 17q may harbor susceptibility genes for recurrent, early-onset major depression.

Genome-wide linkage analysis of lipids in Chinese and Japanese from the SAPPHIRe family study. *C.F. Hsiao¹, Y.F. Chiu¹, L.T. Ho³, Y.J. Hung⁴, K.D. Wu², H.H. Sheu⁵, D. Curb⁷, T. Quertermous⁶, C.A. Hsiung¹.* 1) Biostatistics & Bioinformatics, National Health Research Ins., Taipei, Taiwan, Taiwan; 2) Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan; 3) Department of Medicine, Taipei veterans General Hospital, Taipei, Taiwan; 4) Department of Medicine, Tri-Service General Hospital, Taipei, Taiwan; 5) Department of Medicine, Taichung Veterans General Hospital, Taichung, Taiwan; 6) Stanford University School of Medicine, Stanford, CA; 7) Pacific Health Research Institute, Honolulu, Hawaii.

Lipid levels are recognized as major risk factors for coronary heart disease (CHD). Discovery of major loci underlying quantitative lipid traits could help to elucidate the genetics of CHD. We performed a genome-wide search for quantitative trait loci (QTL) linked to lipid phenotypes in 1538 Han Chinese subjects (509 families) and 625 Japanese subjects (204 families) not taking lipid-lowering medications from the Stanford-Asian Pacific Program in Hypertension and Insulin Resistance (SAPPHIRe) study. The multipoint variance-components method was used to test for linkage between marker loci and each trait by maximum likelihood methods adjusted for linear and nonlinear effects of age as well as effects of gender, BMI, smoking, alcohol consumption, physical activity, and field center. The highest LOD score detected was 3.22 for logarithmically transformed high-density lipoprotein cholesterol (HDL) on chromosome 12 at 113 cM in Han Chinese. This score overlaps the positive findings for HDL reported in Mexican Americans (chromosome 12 at 96 cM). Although no strong evidence for linkage was found in Japanese, some modest peaks were found in several regions that have been reported in other published genome scans. For example, the Japanese SAPPHIRe peaks for HDL (chromosome 3 at 212 cM, LOD=1.51; chromosome 1 at 167 cM, LOD=1.54) were very close to the QTLs for HDL reported in the scans of the Pima Indian subjects (Chromosome 3 at 182 cM), and in the white American HyperGEN (Chromosome 1 at 159.9 cM).

SNP mapping at 100kb-interval in chromosome 21 for localizing the risk loci of late-onset Alzheimer's disease. K.

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Late-onset Alzheimer's disease (LOAD) is one of typical polygenetic disorders, and twin studies in North European countries noted that genetic factors are more important than environmental ones to develop LOAD. Given that patients with Down syndrome develop Alzheimer's pathology at their 30s, we hypothesized that some genes in chromosome 21 harbor the risk effect of LOAD. To search for the risk loci, we performed SNP scanning at 100 kb-interval in chromosome 21 against 188 LOAD cases including 69 cases with definite LOAD and 375 population-based control subjects, using the 5' nuclease assay (TaqMan assay, ABI). This study protocol was approved by Osaka University Genome Committee and the written informed consent was obtained from either subjects or their relatives. Among 417 SNPs, 14 showed allelic linkage disequilibrium with LOAD, judged by p value less than 0.05. One region identified by 3 neighboring SNPs spanning 200 kb was highlighted as a risk locus, and these SNPs also showed a significant effect to modify age-at-onset. This region was located within Down Syndrome Critical Region (DSCR) mapped at 21q22.2, and showed linkage disequilibrium with LOAD also in extended 378 LOAD cases ($p < 0.005$). Although this risk locus should be evaluated in more extended subjects, we propose here that the DSCR harbors a risk locus of LOAD. Our results also indicated that a 100kb-interval scanning against well-defined patients and controls could be effective to screen susceptibility loci of polygenetic disorders.

A Genome Scan for Genes Involved in Vesicoureteral Reflux. *H.R. Kelly^{1,2}, A. Yoneda^{1,2}, D. Shields³, C. Molony^{3,4}, A. Green¹, P. Puri², D. Barton¹.* 1) National Centre for Medical Genetics, Conway Institute for Biomolecular and Biomedical Research and Department of Medical Genetics, University College Dublin; 2) Children's Research Centre, Our Lady's Hospital for Sick Children, Crumlin, Dublin 12, Ireland; 3) Department of Pharmacology, Royal College of Surgeons, Dublin 2, Ireland; 4) Department of Genetics, University of Pennsylvania School of Medicine, Philadelphia, 19104.

Vesicoureteral reflux (VUR), the reverse flow of urine from the bladder into the ureter and kidney, is a common disorder, found in 1-2% of children. VUR can cause kidney damage and is a common cause of end-stage renal failure and severe hypertension in children. It is caused by a shortening of the segment of the ureter that runs through the submucosal layer of the bladder wall. The milder grades of reflux may resolve with time but more severe grades require medical or surgical intervention. VUR occurs frequently in families, suggesting that it is inherited but the mode of inheritance is unknown. Blood from 170 families with more than one child affected with VUR was collected. We are using this resource in an affected-sib-pair approach to search for VUR gene(s). Although no VUR genes have yet been identified, we have excluded all the loci suggested by Feather et al (*Am J Hum Genet* 66:1420-5, 2000). We have recently completed a genome scan using SNP markers across 630 samples to identify the loci involved in VUR. 630 DNA samples from 134 families with >1 child affected with VUR were selected. These families contained 183 sibpairs: 113 sibpairs, 18 sibtrios, 1 sibquad and 1 sibquin. There are 3 sets of twins in the study. Of the 644 samples (630 samples, 14 replicates) sent to Illumina, genotypes were reported on 638 across 4,753 Loci. 3,030,221 genotypes were reported out of a possible 3,032,414 giving a call rate of 99.93%. The mean heterozygosity for the genome scan across 4754 markers was 0.45. Analysis using Genehunter and Merlin indicates the presence of 10 regions with significance p-values <0.01, on 9 different chromosomes. This analysis will map VUR susceptibility gene(s), which will be the first step towards determining the molecular basis of this disorder.

Dissection of the oligogenic susceptibility of familial idiopathic generalised epilepsy. *K.P. Lenzen^{1,2}, A. Heils², G. Rudolf², J.F. Prud'homme², R. Nabbout², O. Dulac², F. Zara², A. Bianchi², R. Robinson², R.M. Gardiner², A. Covanis², B. Koeleman², D. Lindhout², S. Lorenz², P. Nurnberg¹, T. Sander^{1,2}.* 1) Max-Delbrueck-Centrum, Berlin, Berlin, Germany; 2) European Consortium on the Genetics of Idiopathic Generalised Epilepsy.

Idiopathic generalised epilepsies (IGE) affect about 0.3% of the general population and account for 30% of all epilepsies. The aetiology of common IGE syndromes, such as juvenile myoclonic epilepsy (JME) and idiopathic absence epilepsies (IAE), is genetically determined, but the complex pattern of inheritance suggests an oligogenic predisposition. The aim of the present collaborative European genome scan was to map IGE loci and to dissect its oligogenic aetiology. Our linkage study included 90 European families ascertained through a proband with either IAE or JME, and one or more siblings affected by an IGE trait (IGE or generalised spike wave EEG discharges). In total, 561 microsatellite polymorphisms with an average intermarker spacing of 7 cm were genotyped in 377 family members. Non-parametric multipoint linkage analyses, using the GENEHUNTER program, revealed suggestive evidence ($P < 0.01$) for novel IGE loci in the chromosomal regions 5q32 ($Z_{NPL} = 2.87$, $P = 0.002$), 11q13 ($Z_{NPL} = 2.87$, $P = 0.002$) and 13q32 ($Z_{NPL} = 3.51$, $P = 0.0003$). To dissect seizure type-specifying susceptibility loci, two distinct family subgroups were selected by the presence of either myoclonic seizures (JME-families; $n = 38$) or absence of seizures (IAE-families; $n = 52$). In the JME families, maximum evidence for linkage was found in the chromosomal regions 13q32 ($Z_{NPL} = 2.41$, $P = 0.009$), 16q22 ($Z_{NPL} = 2.50$, $P = 0.007$) and 19q13 ($Z_{NPL} = 2.37$, $P = 0.009$). In the IAE families, linkage hints were obtained on chromosome 11q13 ($Z_{NPL} = 3.54$, $P = 0.0002$) and 13q32 ($Z_{NPL} = 3.51$, $P = 0.0002$). Our present linkage results indicate an IGE locus in the region 13q32 that confers susceptibility to a broad IGE spectrum, whereas three additional susceptibility loci on 11q12, 16q22 and 19q13 seem to specify various seizure types in an age-related manner.

Fine mapping and SNP analysis of positional candidates at the pre-eclampsia susceptibility locus, *PREG1*, on chromosome 2.

E.K. Moses^{1,2}, *E. Fitzpatrick*¹, *H.H. Goring*², *H. Liu*¹, *A. Borg*¹, *S. Forrest*³, *D.W. Cooper*⁴, *S.P. Brennecke*^{1,5}. 1) The Royal Women's Hospital, Melbourne, Australia; 2) Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, Texas; 3) Australian Genome Research Facility, Melbourne, Australia; 4) School of Biological Sciences, Macquarie University, Sydney, Australia; 5) Department of Obstetrics & Gynaecology, University of Melbourne, Australia.

Pre-eclampsia/eclampsia (PE/E) is the most common and serious disorder of human pregnancy and is associated with substantial maternal and perinatal morbidity and mortality. A large genetic component to susceptibility to PE/E has been clearly demonstrated and genome scans in Icelandic, Australian/New Zealand and Finnish families have localised putative susceptibility loci to chromosome 2. The locus identified in the Aust/NZ study (designated *PREG1*) spanned a very broad (~ 85 cM) region, with the multipoint LOD-score curve displaying 2 peaks, one on 2p (LOD = 1.96 at 109.2 cM) and one on 2q (LOD = 2.58 at 144.7 cM). Here we describe our fine mapping of the *PREG1* locus with 25 additional STR markers and genetic analysis of two positional candidate genes. Using the robust two-point linkage analysis implemented in the ANALYZE program, all 25 markers gave positive LOD scores with significant evidence of linkage being seen at D2S2313 (151.5 cM) achieving a LOD score of 3.37 under a strict diagnostic model. Suggestive evidence of linkage was seen at D2S388 (107.46 cM) with a LOD score of 2.22 under the general diagnostic model. Two candidate genes beneath the peak on 2p were selected for further analysis using public SNPs within these genes. A maximum LOD score of 3.5 was obtained for a SNP in *TACRI* and 3.33 for a SNP in *TCF7L1*, both achieving genome-wide significance. The STR marker D2S286 for which the Icelandic group obtained their highest LOD score of 4.77 is also within *TACRI*. We therefore suggest that we have identified the same locus on 2p in our Aust/NZ population. However, our significant linkage on 2q is by distance genetically unlinked to the 2p locus and we therefore further suggest that it represents evidence of a separate locus.

Program Nr: 1951 from the 2004 ASHG Annual Meeting

A Whole-Genome Association SNP Panel for Functional Regions. *P.C. Ng, L. Zhou, R. Shen, M.S. Chee, A. Oliphant, K.L. Gunderson.* Illumina, Inc., San Diego, CA.

There are two approaches that have been proposed for whole-genome association studies: the direct approach and the indirect approach. The indirect approach relies on linkage disequilibrium between a marker and a causative variant while the direct approach uses candidate causative variants as markers. The advantage of the direct approach is that fewer SNPs need to be genotyped which results in fewer false positives and lower costs. Moreover, association provides a candidate gene.

To facilitate the direct approach, we have selected approximately 100,000 validated and double-hit SNPs from dbSNP that are located in or near functional regions across the genome. These are assayed using Illumina's whole genome genotyping (WGG) assay on the BeadChip platform. We have concentrated on SNPs in coding regions as well as SNPs in potential regulatory regions. Over 30,000 SNPs lie in transcribed sequences. Those in coding sequences are candidates for affecting protein function and those in UTRs could affect regulation. Another 30,000 SNPs lie in putative regulatory regions. Additional SNPs were chosen so that most exons have at least one marker within 10 kb so that even if the causative variant is not present in the panel, other markers in the panel could be in linkage disequilibrium with the causative SNP. Despite being exon-centric, marker density is relatively homogeneous across the genome. Therefore, the final design is a hybrid that accommodates both the direct and indirect strategies.

By focusing on SNPs in known and candidate functional regions, it is now possible to find disease loci by the direct approach. This panel could also potentially be used for quantitative analysis of allele-specific expression and genomic copy number. Illumina's WGG BeadChip SNP panel enables studies on variation in functional regions throughout the genome.

Examination of chromosomal regions identified in a genomic screen for dementia in Amish families. *S. Prasad¹, J.M. van der Walt¹, S. Slifer¹, P.C. Gaskell¹, W.K. Scott¹, E.R. Martin¹, A. Crunk², D. Fuzzell², M. Creason¹, L. Jiang², K. Spencer², N. Schnetz-Boutaud², K. Welsh-Bohmer³, S.R. Johnson³, C.E. Jackson⁴, C.C. Kroner¹, M.A. Pericak-Vance¹, J.L. Haines²*. 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Center for Human Genetics Research, Vanderbilt University Medical Center, Nashville, TN; 3) Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, NC; 4) Scott and White Clinic, Temple, TX.

We have previously studied the Amish populations in the Midwest and demonstrated a familial effect in late-onset dementia that is not accounted for by APOE. We performed a microsatellite genomic screen in seven complex pedigrees with 123 total sampled individuals (42 affected), using a standard set of microsatellite markers at ~10 cM density. MtDNA analysis indicates that the Amish families originate from three distinct genetic subgroups: Adams County, IN (Group 1), Elkhardt/LaGrange, IN (Group 2) and Ohio (Group 3) (van der Walt et al. ASHG, 2004). In the overall dataset, strongest signals were observed for chromosomes 4, 8, 10 and 20 (Max LOD = 2.16, 2.65, 3.30 and 3.19 respectively). Follow-up analysis included genotyping and analysis of 30 single nucleotide polymorphisms (SNPs) for chromosome 4; 16 SNPs and 3 microsatellites for chromosome 8; 33 SNPs and 2 microsatellites for chromosome 10 and 19 SNPs for chromosome 20. The follow-up dataset included two new families (total affecteds=51). The markers were analyzed under both parametric affecteds-only and non-parametric models using Simwalk2 program. The two-point analyses revealed positive lod scores only on chromosomes 4 (MaxLOD= 1.28) and 10 (MaxLOD=1.04) in overall dataset. Analyses in the Amish subgroups revealed two interesting regions on chromosome 4. Group 1 had a multipoint maximum LOD score of 2.15 at 153Mb and Group 2 had MaxLOD=1.92 at 86Mb. Subgroup analysis did not substantially change the MaxLOD results for chromosomes 8, 10 or 20. These data suggest that multiple loci, one of which is on chromosome 4, may account for risk in the Amish dementia population and may have different effects in different family subgroups.

Genome-Wide Scan for Angiotensinogen Among Nigerian Families Revealed Evidence of QTL on

Chromosomes 1 and 17. *B.O. Tayo¹, X. Zhu², Y. Liang³, A. Luke², P. Muti¹, A.A. Adeyemo⁴, R.S. Cooper².* 1) Department of Social and Preventive Medicine, SUNY at Buffalo, Buffalo, NY; 2) Department of Preventive Medicine and Epidemiology, Loyola Medical School, Maywood, IL; 3) Department of Biostatistics, SUNY at Buffalo, Buffalo, NY; 4) Department of Pediatrics, College of Medicine, University of Ibadan, Ibadan, Nigeria.

Angiotensinogen (AGT) has been intensely studied in the pathogenesis of hypertension over the last several years. As a part of an ongoing family study on the genetics of hypertension in Blacks, we performed a genome-wide scan on 117 Nigerian families comprising of 550 adult members using the software SOLAR to investigate the evidence of possible quantitative trait loci (QTLs) modifying AGT level in this population. We therefore used 378 tandem repeat markers spanning the entire genome with an average map density of 9 cM. The overall heritability of AGT in this population was 76%. Our results of variance component linkage analysis of sex adjusted AGT revealed two positions with genome-wide statistically significant LOD scores of 3.68 on chromosome 17 at 74 cM (between markers D17S2180 and D17S1290), and 3.33 on chromosome 1 at 288 cM (on marker D1S2682) which is close to the position of AGT gene. Other weaker evidences of linkage were found on chromosome 4 (LOD = 2.52) and chromosome 13 (LOD = 2.28). These results suggest the existence of QTLs for AGT on chromosomes 1 and 17 in this black population.

Program Nr: 1954 from the 2004 ASHG Annual Meeting

Hot and cold recombination spots based on the East Finland founder population. *P. Uimari, O. Kontkanen, M. Pirskanen, R. Fuentes, J.T. Salonen.* Jurilab Ltd, Kuopio, Finland.

Linkage disequilibrium (LD) or non-random association of alleles at different loci varies across the genome. In some parts of the genome LD is strong and some regions show weak LD mainly because the area is a hot spot for recombinations. LD was studied in the East Finland founder population that was established some 15 generations ago by 400-800 individuals. The Affymetrix 100k genotyping assay was used for genotyping 118 unrelated individuals. LD was measured for SNPs that were 20-40kb apart from each other. A sliding window approach was used to calculate a mean LD for a 1Mb region with a 0.9Mb overlap between the regions. On average D' was 0.75 for SNPs 20-40kb apart. As was expected, the average D' varied across the genome. As low as $D'=0.2$ was observed for the region in chromosome 12 (113.5Mb 114.3Mb, 12q24.2) including TBX3 gene involved in the regulation of developmental processes; in chromosome 3 (116.2Mb 117.0Mb, 3q13.31) including GAP43 (growth associated protein 43) and LSAMP (limbic system-associated membrane protein); and in chromosome 5 (16.6Mb 17.2Mb, 5p15.1) including MYO10 (myosin). On the other hand, a 2Mb long region in chromosome 8 (77.12Mb 79.1Mb, 8q21.11) indicated very few historical recombinations ($D'0.95$). Totally over 20 regions were observed with an exceptionally high level of LD ($D'0.95$) in regions longer than 0.5Mb.

Program Nr: 1955 from the 2004 ASHG Annual Meeting

A Genome-Wide Scan for Low-Density Lipoprotein Peak Particle Diameter: The Mexican-American Coronary Artery Disease Study. *D. Wang¹, H. Yang¹, M. Quiñones², P. Blanche³, I. Enriquez², X. Jimenez², R. De La Rosa², J. Cadenas², S. Patel², K.D. Taylor¹, W.A. Hsueh², R. Krauss³, J.I. Rotter¹.* 1) Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA; 2) Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA; 3) Children's Hospital Oakland Research Institute, Oakland, CA.

Increased levels of small, dense LDL particles are associated with increased risk for coronary artery disease (CAD). Significant genetic contributions to this trait have been demonstrated by family and twin studies. In order to identify loci that contribute to LDL peak particle diameter (PPD), we performed a genome-wide scan in 82 two-generation Mexican-American families ascertained via a parent diagnosed with CAD. Samples of 360 individuals, including adult offspring and their parents, were genotyped using the Marshfield screen set 12 (380 autosomal microsatellite markers at ~10cM interval). LDL PPD was measured in 235 adult offspring using nondenaturing gradient gel electrophoresis. Heritability was estimated using the variance component method implemented in SOLAR and linkage analyses were conducted using both the variance component method (SOLAR) and the sib-pair regression method implemented in SAGE. The estimated heritability for LDL PPD was 0.85 ($p < 1E-7$) after adjusting for gender, age, and BMI. The strongest evidence for linkage was found on chromosome 17q23 at D17S2193 with a LOD score of 3.1 using the multi-point variance component method and with a p-value of 0.0006 using the sib-pair regression method. This result confirmed the recent finding in the Qubec Family Study. Other suggestive linkages (LOD > 1.3) were also found on chromosomes 1p31, 3q13.1, 8p22, and 14q31. These significant heritability and linkage results suggest a major locus for LDL PPD on chromosome 17q as well as other possible loci contributing to this common CAD risk phenotype.

MSX1 Interacts with PAX9 in Human Tooth Agenesis. *A.R. Vieira¹, R. Meira², A. Modesto³, J.C. Murray¹.* 1) Dept Pediatrics, Univ Iowa, Iowa City, IA; 2) Dept. Pediatric Dentistry, Brazilian Lutheran University, Canoas, RS, Brazil; 3) Dept. Pediatric Dentistry and Orthodontics, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

Individual family studies for tooth agenesis have been used to examine the role of genes but a broader genetic epidemiological approach has not been applied. In this study we sought to determine the association between tooth agenesis and DNA sequence variation in the genes MSX1 and PAX9 in an ethnically diverse human population. Since cleft lip/palate is also associated with both tooth agenesis and the gene TGFA we included TGFA in the analysis as well. Cheek swab samples were obtained for DNA analysis from 116 case/parent trios. Proband had at least one developmentally missing tooth, excluding third molars. Genotyping was performed using SSCP or kinetic PCR assays. Transmission distortion of the marker alleles and DNA sequence analysis were performed. Results showed that tooth agenesis is associated with markers of the genes MSX1 ($p=0.01$) and TGFA ($p=0.01$). No mutations were found in MSX1 or PAX9 coding regions. There was statistically significant data (Table) that suggests MSX1 interacts with PAX9 ($p=0.02$) (Table note: T/NT=transmitted/non-transmitted).

Genes	informative meioses	T/NT	p
MSX1-PAX9	28	10/2	0.02
MSX1-TGFA	23	11/6	0.39
PAX9-TGFA	15	4/1	0.23

These findings suggest that MSX1, PAX9, and TGFA play a role in isolated dental agenesis in humans.

Performance of whole genome amplified samples for microsatellite genotyping. *C. Bark, J. Pettengill, Y. Tsai, P. Boyce, J. Golembieski, J. Gearhart, L. Stewart, M. Zilka, K. Doheny.* Ctr Inherited Disease Research, Johns Hopkins Univ, Baltimore, MD.

Currently the Center for Inherited Disease Research (CIDR) performs microsatellite whole genome linkage scans as a service. For some studies the amount of genomic DNA available is limited. Recently, whole genome amplification of genomic DNA has started to emerge as a viable solution to this problem. In order to evaluate the performance of whole genome amplified DNA samples in microsatellite genotyping, high-quality (lymphoblast-derived) DNA samples were sent to the service labs of Molecular Staging(MS) and Rubicon Genomics(RU). The whole genome amplification (WGA) products were then tested at CIDR using our standard laboratory protocols. Six samples were PCR amplified in 11 independent reactions, resulting in data for 18 markers. Reactions for the control samples(non-WGA) were repeated 4 times, the MS and RU samples were repeated 6 times.

	CONTROL	Molecular Staging	Rubicon Genomics
Genotypes Attempted	414	648	648
% Missing	5.96	2.10	10.54
% Discordant	0	1.61	2.90
% Unbalanced Het	0.26	0.19	6.07

Discordant calls were observed in 2 markers. Discrepancies resulting from the Rubicon products were clearly related to poor morphology of a dinucleotide repeat marker and a single incident of allele drop out. Discrepancies resulting from the Molecular Staging products could have occurred due to unknown laboratory error. In comparing the two WGA methods, the MS derived WGA samples performed better for all three performance measures.

Meta-Analysis of Linkage Studies with BMI: Sum of Lod Scores Analysis. *H. Lyon*¹, *A. Lakhanpal*², *J. Nemes*^{1,3}, *M.J. Daly*³, *J.N. Hirschhorn*^{1,3}. 1) Division of Genetics, Children's Hospital, Boston, MA; 2) Harvard University, Cambridge, MA; 3) The Broad Institute, Cambridge, MA.

Body mass index (weight/height²) is an accurate, heritable and simple measure that correlates with and predicts complications of obesity. Linkage to BMI has been reported in many regions, some of which coincide, but it is not clear which loci, if any, show more evidence of linkage than expected by chance. A combined analysis would be well powered to detect linkage, but raw genotype data are generally unavailable. Methods to combine reported results of multiple linkage studies include parametric methods (e.g. p value pooling, Allison and Heo 1998) or nonparametric methods (Genome Scan Meta-Analysis, GSMA, Badner and Gershorn 2002). GSMA can combine methodologically diverse studies with rank-sum tests to assess significance of linkage at each locus, but this method loses information because each scan's best region is assigned the same rank regardless of LOD score.

We have developed an approach that retains a parametric flavor but allows assessment of significance by permutation. At each genomic location, the test statistic is the sum of LOD scores (SOL) across all studies including only LOD scores ≥ 2 in the calculation to minimize reporting bias. If only p values are reported, we estimate LOD scores = $\ln(p)/2 \cdot \ln 10$. We calculate the SOL at each of 135 bins across the genome. Rather than using arbitrary bins of fixed width, we define bin boundaries using a flexible sliding window to maximize the number of linkage peaks in a bin. To generate a null distribution for assessing significance, we randomly reassign the locations of the LOD scores ≥ 2 and repeat the entire process, including bin definition (10,000 times). To apply this method to BMI, we combined data from 28 linkage studies. We found non-random clustering of linkage peaks in several regions, most notably 3q27. These regions are good targets for candidate gene association studies to identify obesity genes.

Program Nr: 1959 from the 2004 ASHG Annual Meeting

Association study of SPINK5, IL18, and SELP gene polymorphisms with atopic asthma. *F. Belpinati, G. Malerba, E. Trabetti, L. Xumerle, R. Galavotti, P.F. Pignatti.* DMIBG - Biology & Genetics, Univ of Verona, Verona, Italy.

Three genes recently described as associated with atopic asthma have been studied in a sample of 71 North-East Italian family trios with one atopic-asthmatic child. The genes were SPINK5 (serine protease inhibitor - Kazal-type5, chromosome 5q31-33), IL18 (chromosome 11q21-q24) and SELP (p-selectin, chromosome 1q24). The polymorphisms analyzed were: D386N and E420K, 105A/C and V640L in the SPINK5, IL18 and SELP gene, respectively. All the individuals were characterized for clinical asthma, total serum IgE level, skin prick test positivity to common aeroallergens, rhinitis, and bronchial hyperresponsiveness to methacholine. A significant association was found between the SPINK5-E420K polymorphism and clinical asthma ($p=0.014$), atopy ($p=0.014$), IgE levels ($p=0.01$), SPT ($p=0.013$) and rhinitis ($p=0.036$). No association was found for SPINK5-D386N polymorphism, nor for SELP or IL-18 gene polymorphisms and any of the phenotypes. These results are in accord with the association of the 5q31-32 region with allergic asthma phenotypes and suggest that SPINK5 or a closely located gene may play a role in the disease.

Progress of high throughput genetic studies to identify tractable target genes associated with human disease. *S. Chissoe, S. Brewster, D. Burns, B. Caulkins, J. Cogswell, R. DeMent, T. Fleming, D. Hosford, E. Lai, L. Middleton, D. Montgomery, S. Pillai, J. Riley, S. Sehgal, N. Spurr, P. St. Jean, J. Stuart, S. Sundseth, D. Yarnall, A. Roses.* Genetics Research, GlaxoSmithKline, Res Triangle Park, NC, Harlow and Stevenage UK, Upper Merion and Providence, PA.

A significant challenge in the process of developing drugs to address unmet medical need lies in the validation of targets relevant to that disease. Attrition throughout the development process, due in part to intractability, presents further challenges to targets once validated. To address these issues, and to leverage disease understanding with the power of the genome era, a series of high throughput association studies has been initiated.

Three key study components will be described, including the initiation of disease collections, the characterization of tractable genes, and the development of a high throughput infrastructure for sample handling, genotyping and analysis. Collaborations with clinical investigators have been established in twelve disease areas, where rigorous phenotypic data and genetic samples are collected. Through their informed consent for genetic studies, these subjects directly advance focused research aimed at identifying genes associated with human disease. A catalogue of tractable target genes has been assembled, and these genes have been characterized by SNP discovery and mapping. Selected assays are then genotyped against samples of interest, and association analysis performed.

The association study paradigm includes a primary genetic screen followed by a secondary genetic screen to identify confirmed genes. In this way, an enriched pool of targets involved with human disease can be obtained for evaluation. Tractable genes associated with human disease will have a greater likelihood of surviving attrition during the drug development process. These targets also will increase our knowledge of disease pathways that will contribute to hypothesis-driven drug discovery.

Allelic association of the poliovirus receptor-related gene (PVRL2) with the disease course of multiple sclerosis (MS). *S. Gregory*¹, *S. Schmidt*¹, *S. Sawcer*², *L.F. Barcellos*³, *J. Sims*¹, *C. DeLoa*³, *J.R. Oksenberg*³, *S.L. Hauser*³, *A. Compston*², *M.A. Pericak-Vance*¹, *J.L. Haines*⁴. 1) Dept Medicine, Ctr Human Genetics, Durham, NC; 2) Dept Neurology, Cambridge University, United Kingdom; 3) Dept Neurology, UC San Francisco, CA; 4) Ctr Human Genetics Research, Vanderbilt Univ, Nashville, TN.

Multiple sclerosis (MS) is a complex disorder with evidence for genetic influences on both the risk of becoming affected and the disease course. The disease course is highly variable, with some patients not suffering from major disability for several decades post-diagnosis, while others reach wheelchair dependency within a few months or years after onset. We have previously reported association of the APOE-4 allele with severe MS and the APOE-2 allele with a mild disease course. While it is conceivable that APOE may harbor a causal variant that directly influences MS expression, the allelic association may also be due to linkage disequilibrium (LD) with one or more variants in a nearby gene. To test this hypothesis, we have genotyped 40 SNPs within a 0.5 Mb interval around APOE that includes 16 known and novel transcripts, including the poliovirus receptor (PVR), the poliovirus receptor-related gene (PVRL2), and a gene coding for an outer mitochondrial membrane protein (TOMM40). We have assembled one of the largest available data sets for examining MS modifier genes in this region by combining patient data from US and UK cohorts, for a total of almost 1,500 patients and their relatives. Statistical analysis of the 40 SNPs revealed the strongest association with a mild disease course for an SNP within intron 6 of the PVRL2 gene ($p=0.001$). No association with MS risk was found. The association of a viral receptor gene with disease course is consistent with the hypothesis that viral infections may play a role in MS etiology. Molecular work to identify the causal variant responsible for this association is underway.

Replication of the dissection of a human coagulation factor VII QTL. *D.M. Warren¹, S. Cole¹, J.M. Soria², J.C. Souto², J. Fontcuberta², J. Blangero¹, J.W. MacCluer¹, L. Almasy¹.* 1) Southwest Foundation for Biomedical Research, San Antonio, TX; 2) Hospital de la Santa Creu i Sant Pau, Barcelona, Spain.

Coagulation factor VII (FVII) is a vitamin K-dependent protein whose activated form plays a role in the physiological cascade leading to blood clot formation. High levels of FVII have been identified as a risk factor for cardiovascular disease. Recently, the first complete dissection of a quantitative trait locus (QTL) for FVII in humans was conducted using data from Spanish families enrolled in the Genetic Analysis of Idiopathic Thrombosis (GAIT) study. This QTL is located on chromosome 13q, and overlaps the FVII structural gene. Interpretation of the results of that dissection was complicated by the occurrence of two groups of SNPs in complete linkage disequilibrium (LD) that could not be differentiated statistically. One group included four SNPs, and the other, seven. The potential for different patterns of LD in other populations suggests statistical methods may provide an alternative to laboratory-based methods in resolving questions of the individual contributions of each of these SNPs to variation in FVII levels. In order to test this, and to examine further the genetic basis of variation in plasma levels of FVII, we are using maximum likelihood methods to evaluate the FVII QTL polymorphisms examined in GAIT in 522 individuals from 21 Mexican American families participating in the San Antonio Family Heart Study (SAFHS). Preliminary results based on one marker from each SNP group identified in GAIT show FVII levels are associated with two promoter SNPs (-401G/T and -402G/A). In the SAFHS, these two SNPs are in weak LD ($r^2 = -0.274$), whereas they are in equilibrium in GAIT. This suggests we will find a different LD structure in the SAFHS versus that observed in GAIT. We are currently typing the remaining SNPs in this QTL to complete our test of whether a different LD structure in this population will decrease the size of the SNP groups and thereby narrow the number of SNPs requiring laboratory testing.

Characterization of the linkage disequilibrium structure and identification of tagging-SNPs in XRCC4. *K. Allen-Brady, N.J. Camp.* Genetic Epidemiology, Dept. of Medical Informatics, University of Utah, Salt Lake City, UT.

The protein product of the X-ray Repair Complementing Defective Repair in Chinese Hamster Cells 4 (XRCC4) gene (chromosome 5q13-q14) plays an integral role in both the nonhomologous end-joining pathway that functions to repair DNA double-strand breaks in mammalian cells as well as V(D)J recombination, which is essential for diverse immune response. XRCC4 is therefore a popular choice as a candidate gene in association studies for multiple cancer sites. We have characterized the linkage disequilibrium (LD) structure and identified tagging single nucleotide polymorphisms (tSNPs) across this gene in a cohort of unrelated, non-BRCA1/2 breast cancer cases and controls (n=47, n=47) using a principal component analysis (PCA) method [Horne & Camp, *Genet Epidemiol* 2004; 26:11-21]. All SNPs available from Applied Biosystems (ABI) Assays-on-Demand were selected across the XRCC4 gene and genotyped for all subjects. This constituted a total of 21 SNPs at a resolution of approximately one SNP every 10 kb. Genotype completion rate was >99%. From this Caucasian population of subjects, 12 distinct haplotypes with a frequency > 0.01 were estimated using an expectation maximization algorithm. Using the PCA method we identified four LD groups, which captured 97% of the haplotype diversity. The LD groups were mostly contiguous, except for disruption of both groups I and II by two SNPs clearly not in LD with their neighbouring markers. These two SNPs constituted LD group IV by the PCA method. ABI also suggests four LD groups for this gene, but their groups are contiguous and thus they do not capture this disruption of LD structure. The PCA method established that a single tSNP from each group was sufficient to explain >90% of the variance for each group. When analyzed separately, breast cancer cases and controls both gave similar results as the combined analysis, suggesting that affected status does not impact the LD structure of the gene. In conclusion, four SNPs were found to be sufficient to cover >90% of the genetic variation of the total 21 SNPs studied in XRCC4.

Dietary Control Reveals The Association Of Hexokinase 4 (GCK) Polymorphisms With Fasting Glucose. *J. Cui¹, K.D. Taylor¹, X. Guo¹, M.F. Saad², J.I. Rotter¹, R.M. Krauss³*. 1) Medical Genetics, Cedars-Sinai Medical Center, Los Angeles, CA; 2) UCLA, Los Angeles, CA; 3) Children's Hospital Oakland Research Institute, Oakland, CA.

Glucokinase (hexokinase 4, GCK) converts glucose to glucose-6-phosphate and serves as the sensor for glucose in the pancreas. Alternative splicing of GCK results in a longer mRNA specific to pancreatic beta-cells. GCK mutations have been associated with maturity onset diabetes of the young, but the role of GCK variation in glucose metabolism for the general population is unclear. We investigated the relationships between 5 GCK SNPs (mean spacing 6.5 kb) and plasma glucose measurements in 317 healthy male siblings from 144 families given two diets differing in fat and carbohydrate composition. DNA was collected for genotyping from parents and siblings. Blood samples were obtained from siblings after 4 weeks each on isocaloric high fat (HF, 41% of energy from fat) and low fat (LF, 21% of energy from fat) diets in a randomized, crossover study design. Genotyping was performed with TaqMan MGB technology. Using HaploView, a haplotype block composed of 3 SNPs was identified; two SNPs towards the 5-end were each in different block. Associations of individual SNPs and haplotypes in this block were tested with general estimating equations (GEE) that control for familial correlations. Haplotype 4 (H4) of the block was significantly associated with a lower plasma glucose on the HF diet (83.731.13 in subjects with H4 vs 87.010.61 mg/dl without H4, meanSE, $p=0.025$) but not on the LF diet (84.841.35 with H4 vs 85.880.57 mg/dl without H4). The frequency of H4 in founders was 11%. While these SNPs are located in introns, each is located within a region conserved between human and rodents. These results suggest that variation in the GCK gene is important for plasma glucose levels under the condition of a high fat diet. Since GCK forms part of a possible regulatory complex located on the outer mitochondrial membrane, these results are consistent with the recent evidence implicating mitochondrial function in type 2 diabetes, particularly when the beta-cell is subjected to a high level of free fatty acids.

Program Nr: 1965 from the 2004 ASHG Annual Meeting

A haplotype regression model with errors. *C.S.J. Fann, H.-C. Yang.* Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan.

Integration of statistical regression model and haplotype association test not only benefits the adjustments of confounding variable, environmental exposure, risk factor and interactive effect simultaneously, but also provides more information in deciphering complex disorders compared with single locus analysis. Nevertheless, the errors in individual haplotype inferring and phenotypic classification may jeopardize the increased power of haplotype regression analysis. The former attributes to unknown phase of gametic haplotypes and the latter comes from the difficulties in clinical diagnosis or phenotypic definition. We assess the impacts induced by the two kinds of errors and propose a feasible likelihood-based haplotype regression model to formulate the linkage-disequilibrium mapping allowing for errors. We reconstruct individual haplotypes by applying a Bayesian inference using Gibbs sampling. The underlying sampling strategy is the popular case-control study. The inference procedure is also applicable to QTL analysis by considering a generalized linear model. Analyses of authentic data and simulated data are carried out to illustrate and evaluate the proposed method.

Program Nr: 1966 from the 2004 ASHG Annual Meeting

5kb-Resolution SNP map on chromosomes 8q, 9, 18q, 22, and X for the International HapMap Project. *L. Galver, S.S. Murray, A. Oliphant, M. Chee.* Illuminia, Inc, San Diego, CA.

The International HapMap Project is a collaboration among five countries to produce a single nucleotide polymorphism (SNP) haplotype map that identifies common patterns of sequence variation in the human genome (The International HapMap Project, 2003). An initial goal of the project is to generate a SNP map with >5% MAF (minor allele frequency) across the genome with an average spacing of 5 kb. The SNPs will be genotyped on 270 individuals from several populations. We are creating the map for chromosomes 9, 22, X, 8q and 18q, which comprises ~15% of the genome. Approximately 95,000 assays with >5% MAF have been developed so far, and we expect to complete our portion of the 5kb map by September of 2004. Using an average multiplex of 1536 SNP assays in a single reaction (Fan et al., 2003), the data are of high quality with reproducibility, heritability and call rates >99.9%. With the combination of other genotyping centers using the Illumina BeadArray system, >50% data will be generated using the BeadArray™ technology.

We have analyzed the haplotype structures in a CEPH Caucasian sample in three 500kb genomic regions (8q24, 9q34, 18q12) using the methods described in Gabriel et al. (2002). These regions had an average SNP density of 2.9kb +/-0.2kb. All three regions demonstrated similar haplotype block sizes and diversity with an average haplotype block size of 23.2kb +/-3.9kb and an average 4.4 +/-0.3 haplotypes per block. Approximately 67% of the genome was represented in a haplotype block in these three regions. We plan to conduct similar analyses on all of our genotyping data.

We have also analyzed the X chromosome and found distinct boundaries for the pseudoautosomal and homology regions. Both regions have unique population signatures which have enabled us to determine which SNPs lie in these evolutionarily distinctive regions.

Significant association of VLDLR and VEGF in age related macular degeneration. *J.L. Haines¹, L.M. Olson¹, N.C. Schnetz-Boutaud¹, S. Schmidt², W.K. Scott², A. Agarwal³, E. Postel⁴, J.R. Gilbert², M.A. Pericak-Vance².* 1) Ctr for Human Genetics Research, Vanderbilt Univ, Nashville, TN; 2) Ctr for Human Genetics, Duke Univ, Durham, NC; 3) Dep't Ophthalmology & Visual Sciences, Vanderbilt Univ, Nashville, TN; 4) Duke Eye Center, Duke Univ, Durham, NC.

Age-Related Macular Degeneration (AMD) is a significant causes of vision loss in the elderly and has a significant genetic component. To dissect the genetics of AMD, we have assembled a dataset of 165 multiplex and simplex families and a second dataset of 401 cases and 159 examined normal controls. 8 candidate genes were tested based on their potential functional role in AMD. IL1A and CKB were chosen because cDNA microarray experiments demonstrated significantly altered expression in AMD retinal tissue. VLDLR, A2M, LRP6 were chosen because of their known relationship to APOE, which has been consistently demonstrated to modify the risk for AMD. MGST1 was chosen because of its role in oxidative stress, and DCP1 had a previously published association. VEGF was examined because of its role in vascular growth and because it is a target for inhibition therapy in AMD. We used family-based and case-control analyses on multiple SNPs within each gene. Analyses were done using two diagnostic criteria, one including both mild and severe AMD, and the other including only severe AMD. A total of 35 SNPs were tested. A2M and CKB did not demonstrate any association under any analysis. DCP1 generated a single nominally significant SNP ($p=0.03$) only in the family-based analysis. IL1A had two nominally significant SNPs ($p=0.02$; 0.02), LRP6 had three nominally significant SNPs ($p=0.02$; 0.05 ; 0.05), and MGST1 had two nominally significant SNPs ($p=0.02$; 0.03); these results were only found in the case-control analyses. Two genes, VLDLR and VEGF, had nominally significant results in the family-based analyses (VLDLR, $p=0.05$; VEGF, $p=0.001$). These results were replicated in the case-control analyses (VLDLR, $p=0.01$; VEGF, $p=0.02$). While we cannot definitively exclude any of these genes from playing a role in AMD, the replicated positive associations for VLDLR and VEGF suggest that they may play a significant role in AMD.

Protein tyrosine phosphatase *PTPN22* and rheumatoid arthritis. X. Hu¹, S. Schrodi¹, V. Carlton¹, V. Garcia¹, Q. Huang², R. Brandon³, H. Alexander¹, M. Chang¹, K. Ardlie⁴, J. Catanese¹, D. Leong¹, M. Seldin⁶, L. Criswell⁷, D. Kastner⁸, P. Gregersen⁵, E. Beasley³, C. Amos², J. Sninsky¹, A. Begovich¹. 1) Celera Diagnostics, Alameda, CA; 2) University of Texas, Houston; 3) Celera Genomics, Rockville, MD; 4) Genomics Collaborative, Inc, Cambridge, MA; 5) North Shore-LIJ Research Institute, Manhasset, NY; 6) University of California, Davis; 7) University of California, San Francisco; 8) NIH, Bethesda, MD.

We have previously shown that the minor allele of a missense single nucleotide polymorphism (SNP; R620W, rs2476601) in the hematopoietic-specific protein tyrosine phosphatase *PTPN22* is strongly associated with rheumatoid arthritis (Discovery study: allelic $p=6.6E-04$; Replication study: allelic $p=5.6E-08$; Begovich et al. 2004). Bottini et al (2004) have independently shown that the same allele (W620) is associated with type 1 diabetes, suggesting that *PTPN22* may be a key regulator of the immune system. To determine whether *PTPN22* harbors additional causal SNPs/haplotypes, we sequenced the gene in 48 RA patients; to date all but two small exons have been resequenced. We found only one other missense SNP (Q262R, freq = 2%); this SNP was not associated with RA. We then investigated haplotypes in *PTPN22* by genotyping 32 additional SNPs in the discovery sample (475 cases and 475 individually matched controls). Haplo.score (Schaid et al 2002) predicted 10 common (frequency > 1%) haplotypes, only one of which carries the W620 risk allele. A subset of 13 tagging SNPs, identified using the software Redigo (Hu et al. 2004 in press) in combination with biological and statistical information, were used to genotype the replication sample (840 cases in 463 families and 926 individually matched controls). Although there was some heterogeneity between the samples sets, the strongest replicated association was seen for the haplotype containing the W620 risk allele. When we applied a method similar to that proposed by Thomson et al. (1988) and Valdes et al. (1997), we found a second haplotype showed mild evidence for association independent of the R620W SNP. However, our data suggest that the haplotype containing the W620 missense variant is the major risk haplotype of *PTPN22*.

Program Nr: 1969 from the 2004 ASHG Annual Meeting

Accounting for unobserved polymorphisms when evaluating haplotype tagging SNPs. *M.M. Iles.* Medical Epidemiology and Biost, Karolinska Institutet, Stockholm, Sweden.

Haplotype tagging SNPs (htSNPs) are widely used as a means of capturing the majority of the genetic diversity in a region while minimising the amount of genotyping to be performed. Ideally, selection of htSNPs should be carried out using data on all polymorphisms in a region. However, it is usually possible to genotype only a subset of all the SNPs in a region and use these data both to select the htSNPs and judge their efficacy. We demonstrate using publicly available real data that such a strategy leads to an overestimation of htSNP performance and that this bias increases as the observed SNPs become more sparse. At an observed SNP density of 2kb, htSNP analysis suggests that the htSNPs capture on average 95% of the observed variation, when in fact they capture 88% of the unobserved variation. At a density of 10kb htSNP analysis suggests that 93% of the observed variation was captured, when in fact they capture on average only 78%. We propose a method utilising cross-validation to correct for this problem and show that such an approach is both unbiased and more accurate than existing methods. The strategy is applicable both to small-scale studies of a few regions and to large-scale studies of whole chromosomes or genomes such as HapMap.

Program Nr: 1970 from the 2004 ASHG Annual Meeting

Haplotype Association Mapping of Quantitative Trait Loci, A Population Based Approach. *J. Jung, R. Fan, L. Jin.*
Department of Statistics, Texas A&M University, College Station, TX.

In this report, population based regression models are proposed for high resolution haplotype linkage disequilibrium mapping of quantitative trait loci. Suppose that a quantitative trait locus is located in a chromosome region. In the region, a haplotype block is typed which may consist of several markers such as SNPs. Suppose that a population sample is available. For each individual in the population sample, both trait value and genotype at the haplotype block are observed. Two regression models, genotype effect model and additive effect model, are proposed to model the association between the haplotype block and the trait locus. By analytical formulae, we show that the genotype effect model can be used to model the additive and dominant effects simultaneously; the additive effect model only incorporates and takes care of additive effect. The non-centrality parameters of F-test statistics are derived to make power calculation and comparison.

Program Nr: 1971 from the 2004 ASHG Annual Meeting

Evidence for linkage and association to chromosome 19p in autism. *J.L. McCauley, R. Delahanty, T.L. Edwards, L.M. Olson, J.L. Haines, J.S. Sutcliffe.* Center for Human Genetics Research, Department of Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN.

Autism is a complex genetic neuropsychiatric disorder in which affected individuals have life-long disabilities in three core phenotypic domains: language, social relationships, and patterns of compulsive and stereotyped behaviors. Several genomic screens in multiplex autism families have identified numerous loci across the genome, and a few of these (e.g. 7q, 2q) have been replicated across multiple studies. We recently completed a genomic screen in 144 multiplex autism families and identified suggestive evidence for linkage on chromosome 19p13 exhibiting a multipoint heterogeneity LOD (HLOD) of 2.55 at 40cM using a recessive model. To refine linkage information and narrow the linked interval, we genotyped and analyzed single nucleotide polymorphism (SNP) markers at ~1 cM intervals flanking the peak marker D19S930. In addition to narrowing the linkage peak, follow-up association analysis revealed that a marker 5 kb 5' to the CRSP7 gene showed significant association ($P=0.001$) using the PDT test. CRSP7 is subunit 7 of a cofactor required for Sp1 transcriptional activation. To date we have examined 18 SNPs across a 163-kb region inclusive of CRSP7. Several nominally-significant single marker associations have been identified across this large (80kb) linkage disequilibrium block as measured by D' , however no association has been detected in regions immediately flanking the block boundaries. The block spans the CRSP7, SLC35E1, and CHERP genes, in agreement with recent HapMap Project data. Although potential biological relevance of these genes to autism is uncertain, they are positional candidates that may play a role in autism susceptibility.

Identification of significant autism associations on chromosome 7q. D.A. Skaar¹, J.M. Jaworski¹, E.R. Martin¹, A.E. Ashley-Koch¹, J.L. Benton¹, J.L. McCauley², J.L. Haines², J.S. Sutcliffe², G.R. DeLong¹, H.H. Wright³, R.K. Abramson³, M.L. Cuccaro¹, J.R. Gilbert¹, M.A. Pericak-Vance¹. 1) Duke Univ Medical Ctr, Durham, NC; 2) Vanderbilt University, Nashville, TN; 3) University of South Carolina, Columbia, SC.

Multiple studies have indicated that an area of chromosome 7q contributes to autism susceptibility. Microsatellite markers on 7q, d7s1813 (102.82cM), d7s821 (109.1cM), d7s477 (111.79cM), and d7s496 (119cM), have significant linkage. In addition, haplotype and recombination analysis has identified that the region 119.8-122.5cM is predominantly shared among families with autistic individuals. These indications, as well as the location of candidate gene *RELN* in this area, led us to fine-map this region. SNPs approximately 100kb apart and centered on *RELN* (111.3-121.4cM/98.8Mb-108.5Mb) were analyzed in 407 autism families. Both family history positive (fam hx+) and fam hx- families were included. A GGC repeat in the 5 untranslated region (5 UTR) of *RELN* (103.2 Mb), which has been reported as associated with autism, and several other SNPs within *RELN* were also analyzed. The *RELN* markers were also typed on 68 fam hx + families from Vanderbilt University. Significant association was seen for the *RELN* 5 UTR (P=0.002) and the *RELN* Exon 44 SNP (P= 0.03) in the overall dataset. Analysis of the *RELN* 5 UTR and four SNPs found that the most common haplotype for these families was also significantly associated with autism (P=0.002). SNP rs925720, at 98.3Mb, showed significant association (P=0.004) both in the overall data set as well as in sets stratified by family type (fam hx+ or -). There is no significant linkage disequilibrium (LD) seen between this SNP and the *RELN* markers, but rs925720 is in the region spanning the proximal breakpoint of a previously reported paracentric inversion in an autism family ((inv (7) (q22-q31.2)). Significant LD is seen between SNPs within each breakpoint region of the inversion (but not between the two regions). These results continue to support one or more regions on chromosome 7q as contributing to autism susceptibility, and *RELN* is again indicated as a strong candidate gene.

Relation between cumulative relative frequencies of haplotypes and Disequilibrium parameters on the determination of a haplotype block. *M. Tomita*^{1,2}, *R. Takemura*¹. 1) Genome Diversity Team, Integrated Database Group, Japan Biological Information Research Center, Tokyo, Japan; 2) Japan Biological Informatics Consortium, Tokyo, Japan.

On DNA sequences, domain "Hotspot" to which recombination was performed briskly exists, on the other hand, the big domain which there is no recombination not much conversely and is maintaining linkage disequilibrium also exists. It is called "haplotype block". Although the value of D' which is one of the disequilibrium parameters as an important step when determining a haplotype block, 0.9 is used as a thing more nearly experiential than theoretical backing about the threshold value. This report mentions the result which considered the determination of this D' and a haplotype block. The determination method of an old haplotype block is as follows. Since we consider that loci whose the minor allele relative frequency less than 0.1 are the mutation discovered in the comparatively new generation, then does not think it into consideration for the determination of a haplotype block. It decides as the minimum block between loci used as $D' > 0.9$. Although the above is an old determination system, initial conditions are given by considering the domain which is $D' > 0.9$ experientially as the minimum haplotype block, and it is quite determinism-like. Therefore, we investigated the relation between D' and the cumulative haplotype relative frequency. Accumulation of the relative frequency of minor alleles' haplotype of major alleles' haplotype and the value of D' have a very close relation. When a allele relative frequency was 0.5, respectively, that it is 0.95 has proved this cumulative haplotype relative frequency analytically. Moreover, whenever allele relative frequency shifted from 0.5, it also turns out that this relation collapses. Moreover, we also studied the relation between the cumulative relative frequency of three haplotypes and D' . As mentioned above, "unit of haplotype block" and "deviation of haplotype" is defined and the new determination system of haplotype block is proposed. Moreover, this has also been the standard of a new haplotype tagging SNP selection system, and it introduces also.

Significant association and linkage for Alzheimer disease and SNPs in the VR22 and LRRTM3 genes on chromosome 10q. *P.G. Bronson¹, H. Quan¹, R.H. Chung², J. Bartlett³, J.L. Haines³, D. Schmechel¹, G. Small⁴, P.T. Xu¹, J. Gilbert¹, M.A. Pericak-Vance¹, E.R. Martin¹.* 1) Duke Univ. Medical Center, Durham, NC; 2) NC State Univ., Raleigh, NC; 3) Vanderbilt Univ., Nashville, TN; 4) UCLA, Los Angeles, CA.

Linkage studies indicate chromosome 10q contains a gene contributing to Alzheimer disease (AD) susceptibility. The VR22 gene encodes -T catenin, thought to be involved in cell adhesion and interaction with presenilin1. VR22 polymorphisms have been shown to be associated with plasma amyloid protein (A42) levels (Ertekin-Taner et al., 2003), suggesting a possible role in AD. Our previous analysis of 5 VR22 single nucleotide polymorphisms (SNPs) revealed association with late-onset AD (Martin et al., 2003), but these intronic SNPs have no functional significance and a true functional variant remains unidentified. VR22 includes an intron-encoded leucine-rich repeat, which tend to form the amyloid fibrils that compose AD amyloid plaques. Leucine-rich repeat transmembrane neuronal 3 (LRRTM3) is a brain-specific protein expressed in the hippocampus. We genotyped 3 more VR22 SNPs and 3 LRRTM3 SNPs in a large family data set ascertained by Duke Univ., Indiana Univ. and NIMH, and 651 independent cases with 272 unrelated controls.

We conducted association analysis on 319 families with 1,086 discordant sibships. The pedigree disequilibrium test (PDT) yielded significant *p*-values for VR22 SNPs 6 (0.008) and 2 (0.013). The genotype PDT yielded significant *p*-values for LRRTM3 SNP 3 (0.003) and VR22 SNPs 3 (0.039) and 2 (0.049). We also found evidence of linkage, with our strongest results for LRRTM3 SNP 3 (LOD=1.77) and VR22 SNP 5 (LOD=2.29) in the NIMH families (N=349). Our independent case-control data set had significant unadjusted odds ratios that disappeared when adjusting for age at exam and gender. VR22 SNPs 2,3,5 and 6 are in linkage disequilibrium (LD), but LRRTM3 SNP 3 is independent of the VR22 and other LRRTM3 SNPs. Given the biological function of the LRRTM3 gene, our results indicate it is an excellent candidate for further study.

Testing candidate genes on 7q and 16 for autism association. *J.A. Duvall¹, M. Alarcón², J. Simon³, M.G. Arreaza³, K. Wilkes², J.L. Stone¹, S.F. Nelson¹, C. Lese Martin⁴, R.M. Cantor¹, D.H. Geschwind².* 1) Department of Human Genetics, UCLA, LA, CA 90095; 2) Department of Neurology, UCLA, LA, CA 90095; 3) Schering-Plough Research Institute, Kenilworth, NJ 07033; 4) Dept. of Human Genetics, Emory Univ., Atlanta, GA 30322.

Autism is a neurodevelopmental disorder that is characterized by language difficulties, social deficits, and repetitive, stereotyped behaviors. Numerous family and twin studies, reports of autistic individuals with chromosomal abnormalities, and genome-wide linkage scans provide compelling evidence that autism has a significant genetic component. The focus of our present report is on two promising candidate genes, A2BP1 and CNTNAP2 that are currently being investigated in the Autism Genetic Resource Exchange families. A2BP1 was identified via a case report of an affected individual with a chromosomal translocation that disrupts the gene (Martin et al., 2003). Twenty-seven single nucleotide polymorphisms (SNPs), at an average density of 63kb within A2BP1, were genotyped in 212 trios. Two blocks of 3 and 4 SNPs, each, showed nominally significant multipoint transmission disequilibrium (TD), and linkage disequilibrium (LD) within the blocks. The report of the translocation plus this nominal TD and LD, make A2BP1 a promising candidate autism gene. The second gene, CNTNAP2, is a positional candidate identified through a quantitative linkage scan (Alarcón et al., 2002) of autism endophenotypes. Preliminary association analyses of SNPs in 11 genes on 7q including 26 SNPs in this gene suggest that CNTNAP2 is nominally associated with the diagnosis of autism and with language-related quantitative endophenotypes. Interestingly, CNTNAP2 is interrupted in a family with Tourette syndrome and Obsessive Compulsive disorder, both of which share some characteristics with autism (Verkerk et al., 2003). This and the modest evidence for association reported above, make the CNTNAP2 gene a plausible candidate. We are working to confirm the disease-associated polymorphisms in A2BP1 and CNTNAP2 in independent samples, to help elucidate the genetic mechanisms underlying autism susceptibility.

Revealing the role of Glutathione S-Transferase Omega 1 in age-at-onset of Alzheimer Disease. *Y.J. Li¹, W.K. Scott¹, L. Zhang¹, S.A. Oliveira¹, D.E. Schmechel², E.R. Martin¹, J.L. Haines³, J.M. Vance¹, M.A. Pericak-Vance¹.* 1) Ctr for Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Dept of Medicine and Joseph and Kathleen Bryan Alzheimers Disease Research Ctr, Duke Univ Medical Ctr, Durham, NC; 3) Ctr for Human Genetics Research Program, Vanderbilt Univ Medical Ctr, Nashville, TN.

We previously reported Glutathione S-transferase, Omega-1 (GSTO1) associated with age-at-onset of Alzheimer (AD) and Parkinson (PD) diseases. We applied a genomic convergence approach combining genetic linkage, gene expression, and association to demonstrate a role of GSTO1 in AD and PD. SNP7 located in exon 4 of GSTO1 was found to associate with age-at-onset in these two disorders. SNP7 is a non-synonymous change, in which the SNP7-1 allele, the "A" nucleotide coding for Asp, is associated with later age-at-onset. Here, we used an expanded AD dataset to further investigate the role of GSTO1. We confirmed that the evidence of GSTO1 association with age-at-onset is consistent across three different family-based association methods. Same conclusions for GSTO1 remain after adjusting the effect of different ascertainment centers. These results imply that the association of GSTO1 on age-at-onset is not an artifact of a specific statistical method or ascertainment center. Furthermore, we performed the same association analyses on two stratified datasets, the positive and negative linkage subsets, which was based on the family specific LOD score at the peak linkage marker D10S1237. We found that the effects of GSTO1 on age-at-onset come mainly from families with positive linkage indicating that GSTO1 explains the linkage and association signals for age-at-onset. The average age-at-onset difference between GSTO1 SNP7-1 and SNP7-22 carriers in the linkage and association dataset is 6.79 (4.38), much higher than the original 1.57 (7.17) observed in the overall dataset. Thus this variant, which occurs in 33% of the population, significantly delays the age-at-onset of AD within families that share more SNP7-1 alleles.

Identification and Characterization of the Hydrolethalus Syndrome Gene. *L. Mee*¹, *H. Honkala*², *M. Kestila*², *S. Finnila*¹, *I. Visapaa*^{1,2}, *K. Hovanes*¹, *J. Lee*¹, *R. Salonen*³, *G. Jackson*⁴, *L. Peltonen*^{1,2}. 1) Dept Human Genetics, UCLA, Los Angeles, CA; 2) Dept Molecular Medicine, National Public Health Institute and Dept Medical Genetics, Univ of Helsinki, Finland; 3) Dept of Obstetrics and Gynecology, Helsinki Univ Central Hospital; 4) Dept Neurogenetics, UCLA, Los Angeles, CA.

Hydrolethalus Syndrome(HLS) is a recessive lethal malformation syndrome characterized predominantly by hydrocephaly with absent midline structures of the brain. HLS is enriched in the Finnish population with an incidence of 1:20000. Using LD and shared ancestral haplotype analysis of 18 coding SNP markers on 10 candidate genes in the HLS region 11q23-25, we restricted the interval to 525 Kb between PKNOX2 and D11S975. A conserved nucleotide variant was identified in a predicted transcript(Celera:hCG1644899) showing complete association and cosegregation with the disease alleles. Mutation analysis in HLS families revealed that all affected individuals are homozygous for this variant. The carrier frequency of the mutation in 500 control samples was 2.5% in E. Finland, 1.1% in W. Finland, and no carriers were found in the mixed European control panel. Located in the coding region of a novel transcript (HYLS), this A to G mutation results in a D211G change. HYLS has 6 alternative transcripts and is ubiquitously expressed with CNS specificity. Wild- type (WT) HYLS protein in vitro resulted in diffuse cytoplasmic staining whereas immunostaining of the mutant protein revealed distinct nuclear vesicles implying defective cellular targeting of mutant protein also in vivo. WT and mutant transgenic *Drosophila* strains expressing human HYLS were created to identify known gene interacting with HYLS. A WT line was crossed to pan-retinal, pan-neural, ubiquitous, and posterior wing drivers. A wrinkled, crumpled wing phenotype with incomplete penetrance was observed with each driver except the pan-retinal driver. WT HYLS crossed to ubiquitous and posterior wing drivers also produced abnormal bristle polarity and supernumerary bristles in the wing. These results suggest that HYLS could be involved in cell death and/or patterning of sensory organ precursors.

Program Nr: 1978 from the 2004 ASHG Annual Meeting

Association of upstream transcription factor 1 (USF1) with metabolic syndrome and related quantitative traits in Hong Kong Chinese. *M.C.Y. Ng¹, K. Miyaki¹, N.J. Cox², W.Y. So³, V.K.L. Lam³, E.W.M. Poon³, J.K.Y. Li⁴, J.C.N. Chan³.* 1) Departments of Biochemistry & Molecular Biology, and; 2) Human Genetics, University of Chicago, Chicago, IL; 3) Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong; 4) Department of Medicine, Yan Chai Hospital, Hong Kong.

Upstream transcription factor 1 (USF1) regulates the expression of genes involved in glucose and lipid metabolism. *USF1* located at chromosome 1q22-23 is associated with familial combined hyperlipidemia. We and others have previously reported linkage of this region with various metabolic syndrome (MES) traits. This study aims to assess the association of *USF1* with type 2 diabetes (T2D), MES and related quantitative traits in 179 type 2 diabetic families from Hong Kong Family Diabetes Study.

Five haplotype-tagging SNPs (rs790056, rs 3737787, rs2516839, rs2516838, rs1556259) at or near *USF1* were genotyped in 897 family members including 364 males and 533 females. The markers rs790056, rs3737787 and the 5-SNP haplotype were significantly associated with T2D (family-based association tests, $p < 0.01$). The marker rs3737787 and the haplotype showed trends of association with MES ($p = 0.057$). Quantitative trait analyzes revealed that *USF1* was significantly associated with diastolic blood pressure, LDL and fasting insulin in all subjects ($p < 0.05$). Stratification by gender revealed significant association ($p < 0.05$) of these SNPs with total cholesterol and LDL in men, and with diastolic blood pressure in women. Our results suggest that *USF1* may contribute to the development of multiple metabolic abnormalities.

Chromosome 19 and autism: fine mapping, association analysis and phenotypic subsetting. *R. Rabionet¹, D.Q. Ma¹, I. Konidari¹, E.R. Martin¹, A.E. Ashley-Koch¹, G.R. DeLong¹, R.K. Abramson², H.H. Wright², M.L. Cuccaro¹, J.R. Gilbert¹, M.A. Pericak-Vance¹.* 1) Duke Univ Medical Ctr, Durham, NC; 2) University of South Carolina, Columbia, SC.

Evidence from genome-wide screens suggests the involvement of Chromosome 19 in autism. We previously presented the results of our linkage study on 210 multiplex families ascertained through Duke and AGRE (AGRE1)(maximum heterogeneity linkage score (HLOD) of 2.38 at marker D19S593 (17.17Mb)), with the majority of the linkage signal coming from the AGRE1 subset of families. Subsequently, we examined 14 SNPs flanking D19S593 (13.23-21.38Mb) in these data as well as in 200 new families from AGRE (AGRE2). The peak HLOD in the overall dataset was for D19S593 (0.98 (DOM model); 0.78 (REC model)). Again the majority of the linkage information came from AGRE1 (HLOD=3.6 (REC model)). Using the M-test we found significant evidence for heterogeneity showing AGRE1 was significantly different from both Duke and AGRE2 with respect to these data ($p=0.001$). Fine mapping of the region with SNPs located ~ every 100Kb flanking D19S593 using association studies (Pedigree Disequilibrium Test (PDT) and Geno-PDT (genotypic associations)) showed evidence for significant association (AGRE1 dataset) for rs901792 ($p<0.005$; 16.65Mb). Markers rs1870071 (16.37Mb), rs2305777 (13.90Mb) and rs7125 (18.15Mb) also showed significant association ($p<0.05$). Rs901792 also presented significant genotypic association (geno-PDT $p<0.0005$). We also performed phenotypic stratification of our sample by Ordered Subset Analysis (OSA), using as a covariate the presence of obsessive-compulsive traits. OSA analysis of the AGRE1 families increased the multipoint LOD score to 3.294 at 43.15 cM, about 2cM proximal to d19S593. Several possible functional candidate genes are located around these markers: CRSP7, a transcription factor located between rs901792 and rs1870071; EPS15L1, a receptor for the epidermal growth factor near rs1870071; and various predicted genes, like FLJ20241, a putative NFkB activating factor, located near rs2305777. Their involvement if any in AUT susceptibility is currently unknown.

Joint risk due to chromosome 6p variation and exposure to CMV in schizophrenia susceptibility? *B.H. Shirts*^{1,2}, *J.J. Kim*^{1,3}, *M. Dayal*¹, *S. Bacanu*^{1,2}, *J. Wood*¹, *W. Xie*¹, *K.V. Chowdari*¹, *R. Yolken*⁴, *B. Devlin*^{1,2}, *V.L. Nimgaonkar*^{1,2}.
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We previously reported linkage/association with an anonymous marker, M6S125, on chromosome 6p21 among patients showing cytomegalovirus (CMV) exposure. We present data supporting CMV as an infectious risk factor, and report associations with several SNPs on chromosome 6p in CMV positive patients. We used a novel combination of familial aggregation and association studies to investigate the connection of CMV with liability to schizophrenia. We first evaluated exposure to CMV, using serum antibodies, among simplex and multiplex families. Exposure to CMV was significantly increased amongst multiplex families, supporting a connection between CMV exposure and schizophrenia. We also evaluated the role of genetic variation in enhancing risk in CMV seropositive patients by investigating 50 SNPs in 11 genes selected because they localize to chromosome 6p21 and encode proteins mediating CMV infectivity. Comparisons of allele and genotype frequencies in CMV+ and CMV- individuals showed highly significant associations at two SNPs at MICB and one at TNF ($p < 0.005$) indicating that associations at these loci are driven by CMV+ cases. Notably, M6S125 is localized only 6kb from MICB and 71kb from TNF. In preliminary analysis from a replicate of these findings in a separate sample we found significant differences in allele frequencies between schizophrenia patients and controls in six markers at MICB, LTA, and TNF ($p < 0.05$) regardless of CMV status. Interaction of CMV exposure in schizophrenic patients and genetic variation at MICB and TNF is biologically plausible. Our analyses suggest an intriguing interaction between exposure to CMV and variation at MICB and TNF or linked loci in the etiology of schizophrenia. If replicated, these findings would open new vistas of research into gene-environment interactions and schizophrenia pathogenesis.

Quantitative trait loci (QTL) mapping for behavioral traits in recombinant congenic lines of mice. A.

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We used recombinant congenic lines (RCLs) of mice derived from A/J (A background) and C57BL/6J (B background) to map genetic markers associated with the behavioral traits amphetamine (AMPH) induced hyper-locomotion and prepulse inhibition of tactile response (PPI). Both traits are relevant to schizophrenia, a complex psychiatric disorder in human.

We tested 10 animals (on average) for each trait, from 38 congenic and parental lines, informative for 620 microsatellite markers covering the whole genome. Total distance traveled (TDIST) and %PPI were used as indicators of AMPH-induced locomotion and tactile startle response, respectively.

Parental difference for TDIST was significant. We identified lines AcB52 and AcB63 which showed significantly elevated TDIST compared to their parental A/J strain. Markers on chromosomes 1, 9, 10 and 20 were significantly associated with TDIST in these lines. Within the B background, 3 lines significantly deviated from the parental strain. Markers associated with TDIST in these lines mapped to chromosomes 5 and 20.

With respect to tactile PPI there was no significant difference between the parental strains. However, congenic lines A51, A60, B77, B68 and B73 departed significantly from their parentals, and all showed higher %PPI. Markers on chromosomes 1, 2, 4, 6, 8, 10, 11, 17, 18, 19 and 20 were implicated with %PPI in these lines.

The informative lines harboring genes from either A/J or C57BL/6J, which are associated with variation for AMPH-induced hyper-locomotion and %PPI, provide the basis for identifying QTLs involved in these traits. Information from mouse and human genomes along with functional evidences may help to identify suitable candidate genes for behavioral traits relevant to psychiatric disorders such as schizophrenia.

LD and gene expression study of ppp3cc gene in schizophrenia samples. *B. Xu*¹, *E.W.C. Chow*², *A.S. Bassett*², *L.M. Brzustowicz*^{1,3}. 1) Dept Genetics, Rutgers Univ, Piscataway, NJ; 2) Dept of Psychiatry, University of Toronto, and Schizophrenia Research Program, Queen Street Division, Center for Addiction and Mental Health, Toronto, Ontario; 3) Dept of Psychiatry, UMDNJ, New Jersey Medical School, Newark, NJ.

Calmodulin-dependent protein phosphatase 3 (ppp3cc) has recently been reported as a potential candidate gene for schizophrenia that may be responsible for the linkage signal on chromosome 8p21. Because the location of ppp3cc is near to the linkage signal (HLOD = 2.8, alpha=0.9) previously identified in our extended Canadian schizophrenia family, an association study was conducted on the 5 SNPs(cc1a, cc20, cc21, cc33, ccs3) that were reported associated with schizophrenia in the schizophrenia samples. Evidence for LD was assessed using PSEUDOMARKER, which, in contrast to popular TDT-based programs, explicitly separates the evidence for linkage and LD in families with multiple affected individuals. We failed to detect significant association of these SNP markers with schizophrenia in our Canadian samples. Gene expression was also examined with real time RT-PCR to assess the gene expression difference between individuals with schizophrenia and normal controls. Because it is infeasible to directly test gene expression levels in the brain cells of our Canadian samples, two alternative RNA resources were used: lymphocyte cell lines derived from our Canadian samples and brain RNA samples from post-mortem tissue obtained from several brain banks. Reverse transcript PCR indicated that ppp3cc is highly expressed in both brain and lymphocyte cell lines. For the cell lines, there was a trend (t-test p-value =0.12) towards higher expression in the subjects with schizophrenia (group mean = 1.05+/-0.34, n = 9) versus controls (group mean = 0.78+/-0.34, n = 8). Preliminary results with anonymous post-mortem brain samples did not suggest any expression differences, although the family history and 8p21 linkage status of these subjects is entirely unknown. While these initial results do not demonstrate a statistically significant difference in ppp3cc expression in schizophrenia, additional testing with more samples is clearly needed.

Simulational evaluation of effects of multiple testings on chi square statistics in regions with linkage

disequilibrium. *R. Yamada*¹, *H. Kawakami*², *T. Kawaguchi*², *T. Tsunoda*², *K. Yamamoto*¹. 1) Lab for Rheumatic Diseases, SNP Research Center, RIKEN, Yokohama, Kanagawa, Japan; 2) Lab for Medical Informatics, SNP Research Center, RIKEN, Yokohama, Kanagawa, Japan.

Linkage disequilibrium (LD) mapping with high throughput SNP genotyping has been producing promising data to identify disease-susceptible genes for multiple common diseases. Association statistics of LD mapping in those reports were unexceptionally multiple and were always subject to correction for multiple testings. Although in some cases the statistics were perfunctorily corrected by Bonferroni correction, it was apparently too conservative, because polymorphisms in a segment with LD are not independent each other. In other cases permutation test was applied for the purpose and its result was reliable. However permutation test completely depends on observed data sets and application of the test might not be advisable when a large number of data sets are processed. Therefore we simulated regions with LD and assumed to test multiple marker SNPs for association in the regions and evaluated probability distributions of maximal value of chi square statistics that were calculated for case-control association tests on multiple SNPs in a segment with LD. Number of haplotypes was set between two to ten with number of SNPs to be observed up to a couple of dozens. With the increase in number of observed SNPs, the probability distribution of maximal value of chi square statistics from each of the SNPs shifted toward the direction of statistics larger. Although degree of the shift depended mainly on the number of polymorphisms to be tested, the degree was far less than Bonferroni assumption, which was expected. Number of haplotypes affected on the statistics but its effect was smaller. Allele frequency of haplotypes and SNPs, and strength of LD also affected on the test statistics inflation. Simulation-based assessment of effects of polymorphism composition in LD-positive regions on test-statistics inflation will assist precise interpretation of LD mapping data.

A Population-Based Study of CYP11A Haplotypic Diversity and Breast Cancer Risk. *B.L. Yaspan², J.B. Elmore¹, J.P. Breyer¹, K.M. Bradley¹, Q. Cai³, Y-T. Gao⁴, W. Zheng³, J.R. Smith³.* 1) Department of Medicine, Vanderbilt University Medical Center, Nashville, TN; 2) Department of Cancer Biology, Vanderbilt University, Nashville, TN; 3) Department of Medicine and Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, TN; 4) Department of Epidemiology, Shanghai Cancer Institute, Shanghai, PRC.

Cyp11A, the P450 side chain cleavage enzyme (P450_{scc}), is the rate-limiting enzyme of steroid hormone biosynthesis catalyzing the conversion pregnenolone to cholesterol. It is highly expressed in steroidogenic tissues: adrenal, ovary, and placenta. We hypothesized that the CYP11A gene serves as a modifier of breast cancer risk given its central role in estrogen metabolism. We tested the hypothesis in a study population of 1193 breast cancer cases and 1310 age frequency-matched controls from Shanghai, China. To comprehensively evaluate evidence for association with breast cancer we identified all common polymorphism (SNP and STR) within a 46 kb interval harboring the 30 kb gene, and genotyped the study population with those markers efficiently discriminating haplotypic diversity for analysis in a logistic regression framework. Our results demonstrate that the gene resides within a single block of strong linkage disequilibrium represented by 3 common haplotypes, each further divided into several clades. Supporting the hypothesis, a haplotype with a frequency of 10% is significantly associated with breast cancer risk (heterozygote OR=1.45 (95% CI 1.17-1.78), homozygote OR=2.20 (95% CI 1.11-4.34), Chi² P=0.0002). Among the 44 common polymorphisms discovered at CYP11A in Han Chinese, those with alleles uniquely tagging the risk haplotype include two of 3 STRs and a single SNP. Candidate functional polymorphisms are in linkage disequilibrium on the risk haplotype; in vitro tests are underway to provide functional correlates of the breast cancer association.

Pleiotropic effects of the 8.1 HLA haplotype in patients with autoimmune myasthenia gravis and thymus hyperplasia. *C. Vandiedonck¹, G. Beaurain^{1, 2}, M. Giraud¹, B. Eymard³, C. Tranchant⁴, P. Gajdos⁵, J. Dausset⁶, H-J. Garchon¹.* 1) INSERM U580, Paris, CDX 15, France; 2) Association Claude Bernard, Paris, France; 3) Hôpital de la Pitié-Salpêtrière, Paris, France; 4) Service de Neurologie, CHRU de Strasbourg, France; 5) Service de réanimation, Hôpital Raymond Poincaré, Garches, France; 6) Fondation Jean Dausset, Paris, France.

The 8.1 haplotype of the HLA complex has been reproducibly associated to several autoimmune diseases and traits and notably to thymus hyperplasia in patients with acquired generalized myasthenia gravis (MG), an autoantibody-mediated disease of the neuromuscular junction directed at the muscle acetylcholine receptor (AChR). However, the strong linkage disequilibrium across this haplotype has precluded an identification of the causative locus, also termed MYAS1. In the present study, by reconstructing haplotypes and by assessing their transmission in 73 simplex families, we could circumscribe MYAS1 to a segment of 1.2 Mbases maximum size encompassing the class III and the proximal class I region, between the BAT3 and C3-2-11 markers, therefore unambiguously excluding the class II loci. In addition, a case-control comparison disclosed a very strong association of a core haplotype in this same region following an additive model ($p=7 \times 10^{-11}$, odds ratio 6.5 for one copy and 42 for two copies of the core haplotype). Finally, this region was associated with a marked increase in serum titers of anti-AChR autoantibodies ($p=8 \times 10^{-6}$). Remarkably, this effect was suppressed by a second locus in cis on the 8.1 haplotype and located towards the class II region. Altogether the data demonstrate very significant but complex effects of the 8.1 haplotype on the phenotype of MG patients and might shed new light on its role in other autoimmune diseases.

Program Nr: 1986 from the 2004 ASHG Annual Meeting

Parent of origin effect in atopic asthmatic families. *A. Bisognin, G. Malerba, L. Xumerle, E. Trabetti, P.F. Pignatti.*
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A genome wide scan for atopic asthma and related phenotypes was conducted in 123 families of North East Italian descent with two or more asthmatic children. A total of 604 individuals were genotyped for 396 microsatellite markers. The chromosomal regions that showed suggestive linkage were further analysed to explore the hypothesis of a parent-of-origin-effect (genehunter imprinting) in the development of atopy, previously reported in literature especially for dust mite sensitization. Multipoint LOD scores were maximized over disease model parameters using a stepwise gradient strategy both for imprinting and non-imprinting models. A region of chromosome 9 between D9S286 and D9S157, in linkage with dust mite and pet allergen reactivity, showed a highly suggestive evidence of imprinting obtaining a MOD (maximized lod) score of 5.04 under the imprinting model with the following parameters: $f=0.055$ (disease allele frequency), $P(+/+)=0.027$ (penetrance of genotype wild type allele /wild type allele), $P(m/+)=0$ (paternally inherited mutant allele/wild type allele), $P(+/m)=0.3$ (wild type allele/maternally inherited mutant allele) $P(m/m)=0.8$ (mutant/mutant). Under the non imprinting model a MOD score of 2.85 was obtained with parameters $f=0.105$, $P(+/+)=0.01$, $P(m/+)=0.1$, $P(m/m)=0.5$. The difference between the two MOD score values is 2.19. A previous simulation study pointed out that when the difference between the MOD score values of the two models is greater than 1.5, then the higher of the MOD scores indicates the correct mode of inheritance with high reliability. Subject to further confirmation, these data suggest the presence of a new paternally imprinted locus in chromosome 9 between D9S286 and D9S157 possibly involved in the pathogenesis of atopy.

The extent of linkage disequilibrium in the genome of the isolated population of Campora (National Park of Cilento and Vallo di Diano, Italy). *V. Colonna¹, M. Ciullo¹, T. Nutile¹, P. Zavattari², F. Cucca², M.G. Persico¹*. 1) Institute of Genetics and Biophysics A. Buzzati Traverso, CNR, Naples, Italy; 2) Dipartimento di Scienze Biomediche e Biotecnologie, Università di Cagliari, Ospedale Microcitemico, Cagliari, Italy.

This study aims to identify and characterize regions of remarkable linkage disequilibrium (LD) and to assess the relationship between LD and genetic distance in the genome of the isolated inbred population of Campora. This population was selected for the identification of allelic variants responsible of complex traits by means of genomic regions identical by descent. To establish the feasibility of such a study, we have evaluated the extent and position of genomic regions that have not undergone recombination. We have built a pedigree to include Campora inhabitants since 1500 (11,000 members 15-17 generations deep). Current members of Campora population derive from a small number of founders who survived the 1656 plague. We considered a sample of 228 autosomal chromosomes from individuals unrelated up to three generations and belonging to a 2284-member sub-pedigree. Average inbreeding coefficient in this sample is 0.004. We first scanned the Xq13-Xq21 region using 6 STRs markers that have already been used in several populations with different demographic histories and we found, as expected, significant disequilibrium. We then scanned for LD throughout the whole genome, using 1023 STRs markers with an average spacing of 3.4cM. Departure from equilibrium tested by contingency table analysis and evaluation of normalized linkage disequilibrium coefficient (D') was calculated using an improved version of the Haploxt software. Our preliminary results show that 30 regions distributed over 14 autosomes could be in significant disequilibrium within the Campora population. These genomic regions are currently under investigation in order to confirm and to verify the consistence with previously identified regions with similar characteristics in other populations. Finally, a plot of significant p-values against genetic distance suggests extended genomic regions in LD greater than that observed in large outbred populations.

Program Nr: 1988 from the 2004 ASHG Annual Meeting

Linkage Disequilibrium Mapping via Cladistic Analysis: Loss of Information due to Unknown Phase. C.V. Durrant, L.R. Cardon, A.P. Morris. Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford, United Kingdom.

We recently described a method for linkage disequilibrium (LD) mapping, using cladistic analysis of SNP haplotypes in a logistic regression framework (1). However haplotypes are often not known. One possible two-stage approach is to infer the phase of multilocus genotype data and analyse the resulting haplotypes, but inferring haplotypes from phase-unknown data is prone to error. An alternative approach is to analyse the phase-unknown multilocus genotypes themselves, so here we also present an analogous method to that described in Durrant et al. (1) but for multilocus SNP genotypes instead of haplotypes. A simulation study was performed to compare the performance of the genotype and inferred haplotype analyses relative to the analysis of known haplotypes across a wide range of disease models using high-density SNP data based on empirical patterns of linkage disequilibrium. Haplotypes were inferred from the multilocus genotype data using the expectation-maximisation (EM) algorithm and haplotype analysis was performed using the best-guess phase assignment for each individual.(1) C.V. Durrant, K.T. Zondervan, L.R. Cardon, S. Hunt, P. Deloukas, A.P. Morris (2004) Linkage disequilibrium mapping via cladistic analysis of SNP haplotypes. *Am J Hum Genet* 75: 35-43.

Program Nr: 1989 from the 2004 ASHG Annual Meeting

High Resolution T^2 Association Tests of Complex Diseases Using Normal Sibs as Controls. *R. Fan*^{1,2}, *M. Knapp*².
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For late onset complex diseases, parental data are usually not available. One way to study late-onset disorders is to perform sib-pair or sibship analyses. This paper proposes sibship based Hotelling's T^2 tests for high resolution linkage disequilibrium mapping or association study of complex diseases. For a sample of sibships, suppose that each sibship consists of at least one affected offspring and at least one normal sib. Assume that genotype data of multiple markers or haplotype blocks are available for each individual of the sample. Paired Hotelling's T^2 test statistics are proposed for high resolution association study using normal sibs as controls for affected sibs, based on two coding methods: haplotype/allele coding and genotype coding. The paired Hotelling's T^2 tests take not only the correlation between the haplotype blocks or markers into account, but also take the correlation within each sib-pair into account. The validity of the proposed method is justified by rigorous mathematical and statistical proof under the large sample theory. The non-centrality parameter approximations of the test statistics are calculated for power and sample size calculations.

Program Nr: 1990 from the 2004 ASHG Annual Meeting

Cosmopolitan linkage disequilibrium maps. *J. Gibson, W. Tapper, W. Zhang, N.E. Morton, A. Collins.* Genetic Epidemiology Group, Human Genetics Division, University of Southampton, Southampton, United Kingdom.

Linkage maps have been invaluable in the positional cloning of many genes involved in severe human diseases. Standard genetic linkage maps have been constructed for this purpose from the CEPH and other panels, and have been widely used. Now that much attention has shifted towards exploiting linkage disequilibrium and maps of single nucleotide polymorphisms it is of interest to examine the prospects for developing a standard LD map which is somewhat analogous to the linkage map. We have constructed and evaluated a cosmopolitan linkage disequilibrium map by combining data from a small number of populations using data for a 10 MB region on chromosome 20. We find that a cosmopolitan map, which can serve all populations if appropriately scaled, recovers 91-95% of the information contained in the population specific maps. This supports a published pilot study, which examined a number of small genomic regions with a lower density of markers. Recombination hot spots appear to have a dominant role in shaping patterns of LD. The success of the cosmopolitan map may be attributed to the co-localisation of these hot spots in all populations. Although there must be finer scale differences between populations due to other processes (mutation, drift and selection) the results suggest that a whole genome standard LD map would indeed be a useful resource for disease gene mapping.

Program Nr: 1991 from the 2004 ASHG Annual Meeting

Methods to determine the structure of linkage disequilibrium blocks: a comparative study. *A. Guilbot, C. Stalens, I. Elias-Billon, C. Aucan, D. Cohen, F. Macciardi.* Serono Genetics Institute, Biostatistics and Genetic Epidemiology, RN 7, Evry, France.

LD (Linkage Disequilibrium) occurs when two alleles at adjacent polymorphisms are found together in a chromosomal segment more often than expected if they were segregating independently in the population. The extent of LD is influenced by many evolutionary and other confounding factors, but recombination appears to dominate the pattern of LD. Efforts to map oligogenes are concerned with exploiting linkage disequilibrium between markers and putative disease-predisposing loci. So, it is vital to understand the pattern of LD across the genome or candidate region to ensure efficient screening. This task presents some difficulties since block definitions are arbitrary and, by most definition, only a proportion of the genome is composed of blocks. We based our study on a 5 Mb region that we previously characterized by genotyping around 200 SNPs in one case-control study (450 individuals). The LD structure was studied in this sample with four different haplotype-block and haplotype-diversity identification methods. In a second step, we described the underlying structure of LD in the form of a metric map. This alternative approach, assigning locations to markers in linkage disequilibrium units, avoids arbitrary block definition. By comparing the different approaches, we identified some discrepancies in terms of LD block structure clearly related to the differences in block definition. Moreover, we tested the robustness of all methods to Hardy-Weinberg disequilibrium (HWD). The first results showed that, when based on haplotype diversity, the definition of blocks can be influenced by the presence of markers in HWD. On the contrary, the metric LD map remained unchanged and identified the same LD units.

Refinement of the Locus for Myoclonus-Dystonia on Chromosome 18p11 (DYT15). *F. Han¹, D.A. Grimes^{1, 2}, A.E. Lang³, L. Racacho¹, D.E. Bulman^{1,2}.* 1) Dept Molecular Medicine, Ottawa Health Research Inst, Ottawa, ON, Canada; 2) Dept Neurology, Ottawa Hospital, Ottawa, ON, Canada; 3) Dept Neurology, University Health Network, Toronto Western Hospital, ON, Canada.

Inherited myoclonus-dystonia (MD) is an autosomal dominant disorder characterized by alcohol responsive myoclonic jerks typically affecting the arms and axial muscles. Mutations in the epsilon-sarcoglycan (SGCE) gene on 7q21 have been shown to cause MD in about 50% of cases. Previously we excluded SGCE as the cause of MD in a five-generation large Canadian MD family (A). We originally mapped a novel locus for this disorder to a 4 Mb region between the markers D18S1132 and D18S843 on 18p11 (Max LOD score of 3.96). This locus is now designated DYT15 (OMIM number: 607488). Subsequently, a second MD family (B) was shown not to have mutations in SGCE gene and was genotyped with the linked markers on 18p11. Affected individuals in Family B were found to share the same haplotype as that of family A initially for 6 markers in the linked region. Based on the shared haplotype in families A and B we identified the distal recombinant marker as D18S1363. The critical region would have been reduced to 3.27 Mb while the proximal recombinant marker was still D18S843 in these two families. We identified a recombinant SNP in the proximal region of family A and an unshared SNP allele in the distal region. Based on our 20 informative markers, we would expect a shared haplotype to occur at a frequency of less than 8.5×10^{-5} per individual. We have identified a common haplotype on chromosome 18p11 in these two families, refining the position of DYT15 gene to a 2.13 Mb critical region. We have also excluded five candidate genes as the causing genes for MD within or very close to this new critical region.

Program Nr: 1993 from the 2004 ASHG Annual Meeting

New Advances in Association Mapping of Disease Genes Using Linkage Disequilibrium Maps. *N. Maniatis, A. Collins, N.E. Morton.* Human Genetics Division, Southampton General Hospital, University of Southampton, Southampton, United Kingdom.

We have developed a simple yet powerful approach for disease gene association mapping by linkage disequilibrium (LD). This method is unique since it applies a model with evolutionary theory that incorporates a parameter for the location of the causal polymorphism. The method exploits LD maps, which assign a location in LD units (LDU) for each marker. This approach is based on single marker tests within a composite likelihood framework, which avoids the heavy Bonferroni correction through multiple testing. As a proof of principle, we tested an 890 kb region flanking the CYP2D6 gene associated with poor drug metabolising activity in order to refine the localization of a causal mutation. Previous LD mapping studies using single markers and haplotypes have identified a 390 kb significant region associated with the poor drug-metabolizing phenotype on chromosome 22. None of the 27 SNPs were within the CYP2D6 gene. Using a metric LDU map, the commonest functional polymorphism within the gene was located 14.9 kb from its true location, surrounded within a 95% confidence interval of 172 kb. The kb map had a relative efficiency of 33% compared to the LDU map. Despite the low resolution and the strong LD in the region, our results provide evidence of the profound utility of LDU maps for disease gene association mapping. Our findings also indicate that the support interval and location error are smaller than any published results. These tests are robust to large numbers of markers and are applicable to haplotypes, diplotypes, whole-genome association or candidate region studies.

Fine Mapping of Chromosome 11p Reveals Dialectic Interplay Between Genes Controlling Attention and Activity. *L.G. Palacio^{1,2}, F.X. Castellanos^{3,4}, D. Pineda², J.D. Palacio², F. Lopera², R. Long^{1,3}, J.L. Rapoport³, K. Berg¹, J. Bailey-Wilson¹, M. Arcos-Burgos^{1,2}, M. Muenke¹.* 1) NHGRI,NIH,Bethesda,MD,USA; 2) University of Antioquia, Medellin, Colombia; 3) NIMH,NIH,Bethesda MD,USA; 4) NYU Child Study Center, New York, NY, USA.

Genetic polymorphisms at human chromosome 11p have been widely reported to be associated with attention-deficit/hyperactivity disorder (ADHD) and other behavioral disorders. The most extensively replicated reports are for linkage disequilibrium (LD) between ADHD and DRD4. However, the absence of DRD4 mutations, the small effect size ($=1.9$), and the extension of this LD to the DRD4 neighborhood suggest that another locus segregating either in LD, interacting with DRD4, or a common behavioral phenotype might be the real cause of the increased risk for ADHD. We followed up a result of LD between haplotypes at DRD4 and both ADHD and normal population with a ~ 1.5 cM microsatellite screen in extended families from a genetic isolate segregating ADHD. By splitting the phenotype using latent class cluster analysis (LCCA) we found that the strongest LD as obtained by the Pedigree Disequilibrium Test (PDT) occurs between latent classes containing unaffected or normal individuals who significantly exhibit absence of one allele at the marker D11S4046 ($p=0.005$). Using genoPDT, we determined that homozygotes for this allele exhibit increased risk for ADHD. Additionally, we found LD to another allele at this locus conferring protection to develop ADHD ($p=0.04$). We hypothesize that this interplay between antagonistic alleles at the same locus defines the phenotypic class. Because LD at this marker is stronger than for DRD4 and closest to the gene for tyrosine hydroxylase (TH) (~ 200 Mb) compared to DRD4 (~ 2 Mb), TH could be the gene underlying the LD with phenotypes related to activity, attention and comorbidities associated to ADHD. We also found evidence of a population-based block of LD at the telomeric 4 Mb of 11p as additional evidence for hitchhiking of DRD4 by TH. This approach can be tested in other psychiatric conditions, as the LD was detected when using the normal phenotype classes instead of the affected ones.

Follow-up of linkage peaks on chromosomes 1q and 16p for age-related macular degeneration (AMD). M.

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Age-related macular degeneration (AMD) is one of the most significant causes of vision loss in older adults. There is substantial evidence that genes play a critical role in its etiology. To identify susceptibility genes for AMD, several genome-wide linkage screens have recently been completed. Two of the consistent regions of interest across studies are chromosomes (chr) 1q and 16p, which we have explored in more detail. We genotyped 44 SNPs in the 153-177 Mb interval on 1q (density 0.5-1 Mb) and 94 SNPs in the 12-32 Mb interval on 16p (density 100 kb-1 Mb). All SNPs were chosen to have minor allele frequency of at least 10%. Both family-based analysis, using the pedigree disequilibrium test (PDT), and case-control analysis, using logistic regression with adjustment for age at exam and sex, were used to test for single-marker allelic association between these SNPs and AMD. The PDT was applied to a data set of 62 AMD families with 119 discordant sibling pairs, and the case-control analysis was performed on an independent data set of 556 cases and 153 rigorously examined controls. On chr 1q, we detected highly significant evidence for association ($p=0.008$ to 0.0001) in both family and case-control data sets for two SNPs at 170 Mb. On chr 16p, the PDT gave strong evidence for association ($p=0.005$) for two SNPs at 29 and 30 Mb. For both regions, most of the evidence for association came from patients with neovascular AMD. In addition, we continue to see linkage signals in both regions, particularly in families where at least two relatives are affected with neovascular AMD. Additional genotyping in both regions of interest for further localization of putative AMD susceptibility genes is underway.

MTHFR linkage disequilibrium from segregating haplotypes in a Mestizo population from Guadalajara, Mexico.

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5,10-methylenetetrahydrofolate reductase (MTHFR) polymorphisms C677T and A1298C are related to homocysteinemia, folate availability, DNA and protein methylation, and diseases. For these and other polymorphisms at this region, issues regarding selection have been discussed. (Munoz-Moran et al. *Lancet* 352:1120,1998; Rosenberg et al. *AJHG* 70:758, 2002; Zetterberg et al. *Eur J Hum Genet* 10:113, 2002). Linkage disequilibrium (LD) can shed light on some of these aspects. It is usually estimated by maximum-likelihood models. In this study, four MTHFR SNPs (A533G, A1298C, C31T, C677T) were typed by PCR-RFLP (*PaeI*, *MboII*, *AflIII*, *HinfI*) in 200 healthy volunteers from 50 families living in Guadalajara, Mexico. Data from 81 random individuals (162 independent haplotypes) were used to estimate D' (Lewontin *Genetics* 49:49, 1964). Allele frequencies were 83.0% 533G, 20.5% 1298C, 47.0% 31T, 47.0% 677T. Four-site haplotype ² value was 497 (p<0.001). Two-site D' ranges between 0.59-0.91 (minimum distance between sites = 300 bp). D' for (deleterious) haplotype 677T-1298C was -0.85. It has been suggested that over a few generations, folic acid supplementation can modify MTHFR population genetics. This and other selective implications are discussed.

Identification of genes influencing blood pressure variation on chromosome 2p in the Amish. *S. Rutherford, J.R. O'Connell, N.I. Steinle, B.D. Mitchell, A.R. Shuldiner.* Dept Medicine, Univ Maryland, Baltimore, Baltimore, MD.

Unraveling the genetic basis of common complex disorders such as essential hypertension has been complicated by the interaction of environmental influences, genetic heterogeneity and incomplete penetrance. Since the etiology of hypertension begins several years before its clinical manifestation and is a major risk factor for stroke, heart and renal failure, it is important to identify the factors that are likely to have an early influence on blood pressure (BP) determination. Previously, we conducted a genome wide search for BP variation in 691 members of the Old Order Amish population of Lancaster County, Pennsylvania, a genetically homogeneous founder population. A linkage peak for both systolic (LOD = 1.09; $p = 0.01$) and diastolic BP (LOD = 0.46; $p = 0.07$) was obtained in the Amish between 34.8 cM and 86.0 cM on the p arm of chromosome 2. This region shows consistent and convincing evidence for hypertension and BP in other studies. We now report the results of fine-mapping this region with linkage disequilibrium mapping using 26 single nucleotide polymorphism (SNP) markers located in 14 candidate genes spanning 47 Mb. We identified a cluster of 3 SNPs which show significant association with systolic BP ($P = 0.01$ to 0.04) within the genes coding for the brain and reproductive organ-expressed TNFRSF1A modulator (BRE), anaplastic lymphoma kinase (ALK) and latent transforming growth factor beta binding protein 1 (LTBP1). We also observed significant association with diastolic BP ($P = 0.004$ to 0.04) for 2 SNPs within the genes coding for UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 14 (GALNT14) and LTBP1. The associated SNPs constitute a region of less than 5 Mb between chromosome 2p23.2 - 2p22.3. We conclude that the 5 Mb region between 2p23.2 - 2p22.3 is likely to harbor a gene influencing BP variation in the Amish.

Significant Linkage of ADHD and Comorbidities to Loci at 4q13.2, 5q33.3, 11q22, and 17p11: Heterogeneity, Epistasis and Minimal Critical Regions; Lessons From a Genetic Isolate. *M. Arcos-Burgos*^{1,2}, *F.X. Castellanos*^{3,4}, *D. Pineda*⁵, *J.D. Palacio*⁵, *F. Lopera*⁵, *L.G. Palacio*^{1,2}, *J.L. Rapoport*³, *K. Berg*¹, *J.E. Bailey-Wilson*¹, *M. Muenke*¹. 1) NHGRI/NIH/DHHS, Bethesda, MD, USA; 2) Population Genetics, Mutacarcinogenesis and Genetic Epidemiology Group, University of Antioquia, Medelln, Colombia; 3) NIMH/NIH Bethesda MD, USA; 4) NYU Child Study Center, New York, NY, USA; 5) Neurosciences Group, University of Antioquia, Medelln, Colombia.

Attention deficit/hyperactivity disorder (ADHD) is the most common behavioral disorder of childhood. We applied model-based and model-free linkage analyses and the pedigree disequilibrium test (PDT) to the results of a genome-wide scan of extended and multigenerational families with ADHD from a genetic isolate. In these families ADHD is highly comorbid with conduct and oppositional defiant disorders and alcohol and tobacco dependence. We found evidence of linkage to markers at chromosomes 4q13.2, 5q33.3, 8q11.23, 11q22, and 17p11. Minimal critical regions were defined based on recombination events. Fine mapping applied to these regions results in combined significant linkage at chromosomes 4q13.2 (two point allele sharing LOD score from LODPAL = 4.44 at D4S3248), 5q33.3 (two point allele sharing LOD score from LODPAL = 8.22 at D5S490), 11q22 (two point allele sharing LOD score from LODPAL = 5.77 at D11S1998) and 17p11 (two point allele sharing LOD score from LODPAL = 3.73 at D17S1159). Additionally, a suggestive linkage at chromosome 8q11.23 was found (combined two point NPL log (p value) > 3.0) at D8S2332). Several of these regions are novel (4q13.2, 5q33.3, and 8q11.23) while others strongly replicate already published loci (11q22 and 17p11). The significant linkage to different genomic regions in individual families is compatible with the presence of epistasis. Analysis to dissect epistasis using the general model of Olson, and a ~200 Kb resolution fine map using SNP on a second sample of 100 nuclear families from the same genetic isolate is currently underway.

Residual Linkage at NOD2/CARD15 implicates other causal variants at the IBD1 locus. *M.M. Barmada*¹, *J.A. Cavanaugh*², *N. Risch*³, *H. Yang*⁴, *IBD International Genetics Consortium*. 1) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 2) Med Genet Res Unit, ANU Medical School, The Canberra Hospital, Canberra, Australia; 3) Dept Genetics, Stanford University School of Medicine, Stanford, CA; 4) Div Medical Genetics and IBD Center, Cedars-Sinai Medical Center, Los Angeles, CA.

Linkage studies of inflammatory bowel disease (IBD) have unequivocally supported the association between Crohns disease (CD) and the IBD1 locus on chromosome 16 since its identification by Hugot et al. in 1996, and subsequent detection of three common risk alleles (RAs) in the NOD2/CARD 15 locus. These RAs are associated with disease in many Caucasian populations in Europe, the United States and Australia. Nevertheless, the three common alleles (SNPs 8, 12 and 13) do not contribute to susceptibility to disease in either African American or Japanese CD populations studied and questions remain concerning the contribution of known NOD2 RAs to the observed linkage at the IBD1 locus. We have investigated that contribution in the 733 well characterized IBD International Genetics Consortium nuclear families. As expected from previous linkage results, in those CD families in which NOD2 risk alleles are transmitted to affected offspring (N=422), there is strong evidence for linkage, with a maximum nonparametric LOD score ca.19. However, linkage is also observed in the CD families without SNPs 8,12 or 13 RAs (N=213), with a score greater than 3.0. The majority of SNPs 8,12 and 13 RAs occur in association with SNP5 allele 2. While private mutations appear to act as risk alleles in some populations, those detected to date are not usually associated with the haplotype defined by SNP5 allele 2. Functional analysis of SNP5 shows that it does not modify the NF-kB response to MDP in an *in vitro* model, but these data do not rule out altered activity for other functions of the NOD2 protein. We detect evidence of residual linkage at SNP5 (TDT p-value = 0.031, after permutation testing) in families without SNP8, 12, or 13 RAs, suggesting that SNP5 is either in linkage disequilibrium with at least one other causal variant or that SNP5 is itself causal.

Genomewide screen for intraocular pressure in the Beaver Dam Eye Study identifies two novel regions. P.

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Intraocular pressure (IOP) is a principal risk factor for primary open-angle glaucoma (POAG), a leading cause of blindness in the world. In the US, it is estimated that 3 to 6 million people have elevated intraocular pressure (IOP). We performed a non-parametric genome-wide linkage analysis of 218 sibling pairs ascertained through a population-based cohort, the Beaver Dam Eye Study. Detailed medical histories and eye examinations were performed on all participants. IOP was measured with a Goldmann applanation tonometer in each eye. The higher IOP measurement between the eyes was used as a continuous trait, and treatment with drops, systolic blood pressure, sex and age were covariates. Using GENIBD, we estimated the single point and multipoint identity by descent sharing for the sib pairs. Linkage analysis used the modified Haseman-Elston regression models and the option that allows for the non-independence of sib pairs and uses a weighted combination of the squared trait difference and squared mean corrected trait sum in SIBPAL (S.A.G.E. version 4.5). For each marker, p-values were obtained using the asymptotic distribution of the likelihood-ratio test statistics. The two-point whole-genome scan yielded two novel loci on chromosome 6 (D6S1027, $p = 0.008$) and 13 (D13S317, $p = 0.0007$), as potential linkage regions. Neither locus has been identified previously in genome-wide screens for POAG. Multi-point linkage analysis weakened evidence for linkage in both regions. Neither region reached genome-wide significance. The chromosomes 6 and 13 regions warrant further attention; especially since neither of these loci has been previously identified in genome wide scans of POAG. It is plausible that IOP, like blood pressure, is heterogeneous and there may be loci that control the pressure in both the eye and blood. A whole genome linkage analysis is currently underway using the complete family structure from the Beaver Dam Eye Study (2231 individuals).

A genome-wide scan of episodic vertigo and migraine: Suggestive linkage to 2p11-14 and 17q23-25. *H. Lee¹, H. Wang², J.C. Jen³, C. Sabatti^{1,2}, R.W. Baloh^{3,4}, S.F. Nelson^{1,5}*. 1) Dept. Human Genetics, UCLA, Los Angeles, CA; 2) Dept. Statistics, UCLA, Los Angeles, CA; 3) Dept. Neurology, UCLA, Los Angeles, CA; 4) Dept. Surgery, UCLA, Los Angeles, CA; 5) Dept. Psychiatry and Biobehavioral Sciences, UCLA School of Medicine, Los Angeles, CA.

Vertigo is a perception of movement, either of oneself or of objects around one. It is caused by lesions of the vestibular system (from inner ear to brain pathways). Vertigo is commonly reported in patients with migraine (with or without visual aura) but there is little understanding of the molecular basis of migraine associated vertigo. It is generally accepted that there is a strong genetic component for migraine although preliminary linkage studies suggest genetic heterogeneity. There have been no prior linkage studies in families with migraine and episodic vertigo. We performed a genome-wide linkage scan in 22 families in which multiple members had episodic vertigo and migraine headache, with or without visual aura. With the Affymetrix 10K SNP Mapping Assay, we could perform rapid, accurate and efficient genotyping across the whole genome at a median intermarker distance of 105kb. We performed parametric (heterogeneity LOD [HLOD]) linkage analysis under an autosomal dominant model and nonparametric (S(all) statistics) linkage analysis as well. We report suggestive linkage on 2p11-14 (HLOD=2.9, NPL=2), and 17q23-25 (HLOD=2.5, NPL=2.4). Additional regions with evidence of suggestive linkage were observed on 5p14 (HLOD=2.3, NPL=1.5), 10q25-26 (HLOD=2.2, NPL=1.2), 16q12-21 (HLOD=2, NPL=2). We plan to test additional families to verify these suggestive regions but it appears that migraine associated vertigo is heterogenetic like other migraine syndromes.

Bivariate genome scan analysis reveals a new locus for obesity traits in Mexican American Coronary Artery

Disease (MACAD) families. X. Li¹, M.J. Quinones², D. Wang¹, I. Enriquez², X. Jimenez², R. De La Rosa², J.G. Cadenas², D.J. Bustos², W. Hsueh², J.I. Rotter¹, H. Yang¹. 1) Medical Genetics Institute, Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) UCLA, Los Angeles, CA.

Obesity is one of the most important components of the metabolic syndrome. Genetic factors are involved but the mechanism and magnitude of common genetic effects are less clear. Using univariate genome scan linkage analysis, we previously reported evidence for linkage at chromosome 2q36-37 for body mass index (BMI), body surface area (BSA), and trunk fat mass as percent of body weight (FM-T%) (LOD= 2.35, 3.16, 2.62, respectively) and at chromosome 5q36 (LOD=2.18, 2.06, 1.00, respectively) in MACAD families. To identify potential loci with a pleiotropic effect on obesity traits, we evaluated the genetic correlations between traits and performed a genome-wide linkage analysis using bivariate models in 443 subjects from 101 Mexican American nuclear families ascertained through a proband with documented coronary artery disease, the MACAD study. Heritability estimates and multipoint linkage analysis were performed using a variance components procedure implemented in SOLAR. After taking into account age and gender effects, we observed significant genetic correlations between BMI and other obesity related traits (all $p < 0.01$). These significant correlations indicated that the pleiotropic effects of common genes account for 0.36 to 0.50 of genetic variance in these trait pairs, with the highest for BMI-BSA ($G^2 = 0.5$) and lowest for BMI-WHR (waist hip ratio) ($G^2 = 0.36$). Bivariate linkage analysis identified a new peak (LOD=3.25) at 25cM on chromosome 7 for the pair of BMI and BSA. These results indicate that 1) the pleiotropic effects of both genetic and environmental factors contribute to the strong correlation between BMI and other obesity traits, especially BSA, 2) the prior evidence for univariate linkage on chromosomes 2q36-37 and 5q36 was supported by bivariate analysis, and 3) an additional linkage peak at 7p15 was identified by the bivariate model. This suggests that the use of bivariate models provides additional power to identify linkage of genes responsible for obesity related traits.

Novel evidence of RP1 mutations causing autosomal recessive retinitis pigmentosa in consanguineous Pakistani families. *S.Q. Mehdi¹, K. Anwar¹, A. Abid¹, M. Ismail¹, A. Hameed¹, P. Lall², Amtul-Aziz³, S. Khaliq¹.* 1) Biomedical and Genetic Engineering Laboratories, Islamabad, Pakistan; 2) Christian Hospital, Faisal Shaheed Road, Taxila, Pakistan; 3) Al-Shifa Trust Eye Hospital, Rawalpindi, Pakistan.

The second most common cause for adRP, after rhodopsin dysfunction, is mutations in the gene for RP1, and are responsible for approximately 6-10% of all adRP in ethnically different populations. More than 35 mutations in the RP1 gene that cause adRP, are mostly clustered in exon 4 and result in premature termination of the protein. However, RP1 has not been reported to cause any other form of retinal degeneration. In this study we analyzed three consanguineous arRP Pakistani families. Exclusion studies of known RP loci showed homozygosity with markers D8S285 and D8S1815 flanking the RP1 gene locus. Heteroduplex analysis for RP1 gene using DHPLC revealed heteroduplex peaks in exon 4 of all the unaffected parents. All family members were then sequenced to identify the mutation in exon 4. In family 442RP and 452RP, a homozygous C to T missense substitution in codon 373 (ACA to ATA; T373I) was found in all the patients, while parents of the affected individuals and some of the normal siblings were heterozygous. None of the 100 normal controls carried the T373I change in the homozygous state, although some heterozygous individuals were observed. In family 336RP, a 4-bp insertion at nucleotide 1461 adds a termination codon after codon 487. This results in the production of a severely truncated protein of 487 instead of 2156 amino acids. This was homozygous in all patients, heterozygous in the parents and some normal family members, but not in normal controls. A random panel of 150 Pakistani RP patients was screened. A second missense G to A substitution (GCC to ACC; A669T) was found in a heterozygous state in one patient but not in the normal controls. These results suggest that the frequency of RP1 gene mutations is relatively low in Pakistan. To our knowledge this study provides the first report of the involvement of mutations in the RP1 gene in the autosomal recessive RP phenotype.

Fine mapping of the PDB4 locus at 5q31. *J. Morissette¹, S. Auger¹, A. Duchesne¹, N. Laurin², J.P. Brown³.* 1) Dept Molec Endocrinology, CHUL Research Ctr, Quebec, PQ, Canada; 2) Cell and Molecular Division, Toronto Western Hospital, Toronto, ON, Canada; 3) Rheumatology-Immunology, CHUL Res Centre, Quebec, PQ, Canada.

Paget disease of bone (PDB) is characterized by focal increases of the bone remodeling process. It is the second most common metabolic bone disease after osteoporosis. Genetic factors play a major role in PDB and seven loci have been reported so far. Only the PDB3 locus, localised at 5q35, has been confirmed and the gene identified. A recurrent mutation (P392L) in the exon eight of the sequestosome 1 (SQSTM1/p62) gene was detected in affected members of 11 of 24 (46%) French Canadian pedigrees and in 27 of 182 (15%) unrelated French Canadian affected individuals. A second locus, PDB4, was also localized within the same population. A localization interval of 12 cM at 5q31 was defined between markers AFM286xg9 and AFMa190xb1 using two large multigenerational families. A different disease haplotype was observed in those two families.

To further refine the PDB4 locus, we exploited the founder effect of the French Canadian population. We genotyped a total of 28 informative microsatellite markers in 12 families, 130 unrelated affected individuals and 143 individuals sampled from the general population. Ten of those markers were developed locally using the human genome sequence. Haplotypes from family members were phased using the SIMWALK V2.8 program whereas the haplotypes of unrelated individuals were phased with the PHASE V2.0 program.

Portions of the two PDB4 linked family haplotypes were found in unrelated individuals. This allowed us to narrow the PDB4 interval within a 4 cM region. This PDB4 region is now defined by the two markers AFMa103za9 and GATA2H09.

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A highly informative SNP linkage panel for human genetic studies. *S.S. Murray, B.G. Kermani, R.J. Steeke, S.G. Shannon, A. Oliphant, M.S.T. Hansen.* Illumina, Inc, San Diego, CA.

We have developed a highly informative set of SNP assays designed for linkage mapping of the human genome. These assays were developed on a robust multiplexed system to provide an unprecedented combination of very high accuracy, data completeness, and high throughput for linkage studies. The performance of the system together with objective, quantitative metrics of data quality effectively eliminates the need for labor-intensive post-processing of datasets prior to linkage analysis. The linkage panel is comprised of approximately 5600 SNP loci with an average ~570 kb spacing. The average minor allele frequency for these SNPs is approximately 0.40 in a Caucasian population. Allele frequencies are also being determined in Asian and African populations. Data quality has been shown to be exceptional, with data quality metrics (call rate, reproducibility, and genotypes consistent with Mendelian inheritance) routinely >99.9%. We have also determined the relative genetic map position of each SNP locus by interpolation to a high resolution genetic map (Kong et al., 2002), where the mean genetic map interval is 0.6 cM. In addition, the relative information content of this panel is higher than the 5- and 10-cM commonly used STR marker panels tested, and is >0.95 for all chromosomes using 188 meioses derived from 8 CEPH reference pedigrees. Information content is also currently being estimated from simulated genotype data using the allele frequencies from the Asian and African populations to determine the usefulness of this panel in these populations. The potent combination of this SNP linkage panel with the multiplexed assay system provides a previously unattainable level of performance for linkage studies.

STRATIFICATION REVEALS SIGNIFICANT SEX-SPECIFIC LINKAGE TO ATTENTION-

DEFICIT/HYPERACTIVITY DISORDER (ADHD). *M.N. Ogdie¹, S.E. Fisher², M. Yang³, C. Francks², J.T.*

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Genome-wide linkage analysis of a sex stratified sample of 308 ADHD affected sibling pairs (ASPs) indicates the presence of sex-specific susceptibility loci. A region on 5p13 (MLS 5.13, empiric $p=0.02$) yielded significant linkage to ADHD only in male sibling pairs. Simulations were performed to assess the statistical significance of observing the increase in linkage signal across the genome. Analysis of the overall genome-wide increase in evidence of linkage in the stratified samples strongly argues the presence of multiple sex-specific effect loci (empiric $p10^{-6}$). The identification of multiple distinct linkage regions in both male and female ADHD ASPs suggests that linkage analysis of sex stratified samples should be routinely conducted in ADHD linkage studies.

Evidence for Glucose Homeostasis Genes in African Americans on Chromosome 11q: The IRAS Family Study.

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Disposition Index (DI) is a quantitative measure which combines the effects of insulin sensitivity (S_I) and β -cell function (AIR) in an assessment of glucose homeostasis. We previously reported linkage of DI on chromosome 11q with a LOD of 2.61 at 67cM (LOD-1: 63-99cM) in 284 African Americans (21 pedigrees) from the IRAS Family Study. This finding was confirmed in an additional 214 African Americans (22 pedigrees) from IRASFS with a LOD of 1.83 at 60cM (LOD-1: 38-92cM). Fifteen additional SSTR markers were added to this region, resulting in an increase in the combined LOD score (LOD=3.95; 80cM) and refinement of the peak (LOD-1: 74-84cM). AIR also showed evidence of linkage to two separate regions (LOD=3.08; 64cM and LOD=2.73; 85cM) distinct from the DI peak. In contrast, S_I was not linked to this region. Information content for these markers suggests little additional linkage information could be obtained by adding markers. As S_I , AIR, and DI are mathematically and metabolically related, bivariate linkage analyses were performed. These analyses did not improve or refine the linkage signals. Thus, these data do not strongly support a hypothesis of locus-specific pleiotropy between S_I , AIR, and DI. Nine potential candidate genes in this region were evaluated for association to DI and AIR. Among these candidates, muscle glycogen phosphorylase showed the most significant associations with DI ($P<0.001-0.011$) and pyruvate carboxylase showed the most significant associations with AIR ($P<0.001-0.002$). To summarize, our African American pedigrees confirmed linkage evidence for the 11q DI locus and identified an independent AIR loci on 11q. Separate positional candidate genes provide evidence of association with DI and AIR.

Identification of a novel locus on chromosome 2q for autosomal dominant high myopia. *P.C. Paluru¹, S. Nallasamy^{1,2}, M. Devoto³, S. Brown⁴, T.L. Young^{1,2}*. 1) Department of Ophthalmology and Genetics, Children's Hospital of Philadelphia, Philadelphia, PA; 2) University of Pennsylvania School of Medicine, Philadelphia, PA; 3) Department of Biomedical Research, Nemours Children's Clinic, Wilmington, DE; 4) Department of Ophthalmology, Texas Technical University Health Sciences Center, Lubbock, TX.

Myopia, or nearsightedness, is a visual disorder of high and growing prevalence in the U.S. and in other countries. "Pathologic" high myopia, or myopia of -6.00 diopters (D), predisposes individuals to retinal detachment, macular degeneration, cataracts, and glaucoma. Autosomal dominant (AD) non-syndromic high-grade myopia has been mapped to loci on chromosomes 18p11.31, 12q21-23, 17q21-23, and 7q36. We now report significant linkage to a novel locus on chromosome 2q in a large, multigeneration family with AD high myopia. The family contains 30 participating members (14 affected). The average spherical refractive error for affected individuals was -14.15 D (range, -7.25 to -27.00). Linkage to intragenic or flanking markers for the myopic genetic syndromes of Stickler syndrome types 1, 2, and 2B; Marfan syndrome; Ehler-Danlos syndrome type 4; and juvenile glaucoma was ruled out before proceeding with a genome screen. In addition, no linkage was found to the known AD loci listed above. A full genome screen of the family was performed with 382 microsatellite markers with an average inter-marker distance of 10 cM. SimWalk2 software was used for multipoint linkage analysis based on an AD model with a penetrance of 0.9 and a disease allele frequency of 0.001. Additional fine-point mapping yielded a maximum multi-point LOD score of 5.606 at marker D2S2348. Haplotype analysis defined a critical interval of 10.37 cM on chromosome 2q37. A novel locus for AD high-grade myopia has been determined, providing further evidence of genetic heterogeneity for this disorder.

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A new locus for autosomal dominant posterior polar cataract on chromosome 14q. *E. Pras, J.F. Hejtmancik.*
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Background: Posterior polar cataract is a clinically distinctive opacity located at the back of the lens. It is commonly acquired in age related cataract, and may infrequently occur in pedigrees with congenital cataract. To date, four loci for autosomal dominant congenital posterior polar cataract have been identified. These include one gene CRYAB on chromosome 11, and three loci with as yet unknown genes on chromosomes 1p, 16q and 20p.

Purpose: To find the chromosomal location of a gene causing autosomal dominant congenital posterior polar cataract in three Morocco-Jewish families.

Methods: A whole genome scan was performed using microsatellite markers spaced at approximately 10cM intervals. For fine mapping 4 additional microsatellite markers were genotyped. Two-point lod scores were calculated using MLINK of the LINKAGE program package.

Results: The new cataract locus was mapped to an 11.2 cM interval between D14S980 and D14S1069 on chromosome 14q. A maximum two-point lod score of 3.67 at $\theta=0$, was obtained with the markers D14S274.

Conclusion: A gene associated with posterior polar cataract maps to the long arm of chromosome 14.

A QTL on chromosome 8q influences the skeletal maturation of healthy children during early and middle childhood. *B. Towne¹, D.L. Duren¹, J. Blangero², J.S. Parks³, M.R. Brown³, T. Dyer², S.A. Cole², A.F. Roche¹, R.M. Siervogel¹.* 1) Wright State University School of Medicine, Dayton, OH; 2) Southwest Foundation for Biomedical Research, San Antonio, TX; 3) Emory University School of Medicine, Atlanta, GA.

Current understanding of genetic influences on skeletal development comes mainly from studies of monogenic disorders. Few studies have examined genetic influences on normal variation in skeletal development. The tempo of skeletal maturation in healthy children is variable, with the skeletal age (SA) of a child differing up to three years from that child's chronological age. Here, we present results from an initial series of linkage analyses of the SA of children assessed at chronological ages 1 to 17 years. SA data were obtained from 9,865 hand-wrist radiographs taken between 1931 and 2002 of 1,069 children from 220 families in the Fels Longitudinal Study (Roche, 1991). SA was estimated by the FELS method (Roche et al., 1988), a maximum likelihood approach that uses measured and graded indicators of skeletal development appropriate for a given chronological age. An initial set of 478 subjects has been genotyped for some 400 autosomal markers spaced approximately every 10 cM. A variance components-based linkage analysis method (SOLAR; Almasy and Blangero, 1998) was used to analyze these data and obtain multipoint LOD scores. SA was highly heritable throughout childhood, and we found consistent evidence of linkage of SA at chronological ages 2 to 10 years to chromosome 8q between markers D8S270 (8q21.3) and D8S1784 (8q23.1). The LOD scores across these nine ages were: 1.08, 0.96, 1.55, 2.01, 2.76, 1.43, 3.16, 2.06, and 0.99, respectively. Disorders with skeletal abnormalities as part of their etiology mapped to 8q22-q24 include Klippel-Feil syndrome and Langer-Giedion syndrome. Also, the bone morphogenetic protein 1 (BMP1) gene at 8q21 is within the support interval of this study. Our future work will seek to identify specific genes and polymorphisms in this region that influence different aspects of normal skeletal development during early and middle childhood. Supported by NIH grants HD36342, HD12252, and MH59490.

Fine mapping of the keratoconus locus on 16q. *S. Tuupanen*¹, *P. Sistonen*², *T. Tervo*³, *T. Alitalo*¹. 1) Biomedicum Helsinki, Department of Obstetrics and Gynecology, Helsinki University Central Hospital, Finland; 2) Finnish Red Cross, Blood Transfusion Center, Helsinki, Finland; 3) Department of Ophthalmology, Helsinki University Central Hospital, Finland.

Keratoconus (KC; MIM 148300) is a noninflammatory corneal disorder with an incidence of approximately 1:2000. KC is characterized by progressive thinning, protrusion and scarring of the central cornea, leading to variable degree of visual impairment. It is the most common cause of corneal transplantation in Western world. We have previously performed a genome-wide linkage analysis and localized KC to 16q22.3-23.1 in 20 Finnish families. Based on haplotype analysis, we mapped the gene between markers D16S2624 and D16S3090, an interval of 6,9 cM. Each family had their own disease haplotype in this region.

The aim of this study was to fine map the 6.9 cM region with single nucleotide polymorphisms (SNPs) to narrow down the candidate gene region. We collected SNPs from public databases, concentrating first on known genes and later expanding the SNP map to span over the whole genomic region. The most promising candidate genes were covered with a tighter marker map. We have presently genotyped 78 SNPs, with an average density of 66 kb, in our family material using a SNP micro-array method. Association in the presence of linkage was tested by FBAT and Pseudomarker but none of the SNPs has so far showed significant association. This could be due to small family material and incomplete penetrance of the disease gene. On the other hand, haplotype analysis has revealed a 462 kb haplotype block where four different haplotypes, shared by 14 families, can be observed.

Based on these preliminary results we can conclude that there must be more than one mutation responsible for KC in Finnish families. To ascertain the association of the 462 kb block to KC, we have now genotyped 100 control samples with 66 of the 78 SNPs. Analysis is in progress to determine the haplotype block structure in our candidate gene region in the Finnish population.

Linkage analysis using GeneChip Microarray maps new locus for microcephaly to chromosome 12q24. V.

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We report a large Israeli Arab family with multiple consanguineous marriages in which 9 children have congenital microcephaly associated with severe mental retardation, spasticity and hyperreflexia. Dysmorphic features are mild and non-specific. A dense genomewide linkage search of the family was undertaken using Affimetix GeneChip Human Mapping 10K array. This microarray contains over 10,000 SNPS and therefore allows efficient genotyping. Initial analysis was performed on DNA from 4 affected family members. The results showed 5 different regions with homozygosity for the same allele in more than 10 loci in a row. These regions were further analyzed using polymorphic markers in affected and unaffected family members. Four of these loci were excluded. One locus on chromosome 12q24 was identified as a possible candidate locus for the gene. This interval contains homozygous SNPs and is 8.5Mb long. Haplotype analysis using different markers within this region narrowed the locus down to 6.2 Mb with a maximum lod score of 3.4 (= 0.00) at marker D12S837. This study identified a new locus responsible for microcephaly located on chromosome 12q24. Furthermore we demonstrated that GeneChip Microarray SNPs is an efficient technique in linkage analysis of inbred families with autosomal recessive diseases.

Identification of new dinucleotide-repeats in factor gene using fluorescent PCR. *J.W Kim¹, S.Y. Park¹, D.J. Kim¹, S.Y. Kim¹, J.Y. Han², H.M. Ryu^{1,2}.* 1) Laboratory of Medical Genetics, Samsung Cheil Hosp, Seoul, Korea; 2) Department of Obstetrics and Gynecology, Sungkyunkwan University School of Medicine, Seoul, Korea.

Hemophilia A is an X-linked inherited bleeding disorder, and linkage diagnosis using polymorphic markers in the factor gene is used to archive the carrier detection and prenatal diagnosis. The objective of this study was to establish the allele frequency and heterozygosity rate (HR) of two new (Intron 1, and 24) and other intragenic markers (Intron 13, and 22) using fluorescent PCR. Five-hundred unrelated healthy women were screened, and a hemophilic family was studied for carrier detection and prenatal diagnosis. We observed five different alleles of Intron 1, 10 of Intron 24, 9 of Intron 13, and 6 of Intron 22. The observed HR for the Intron 1, 24, 13, and 22 were 34.0 %, 35.2 %, 53.0 %, and 42.6 %, while the expected HR were 33.6 %, 36.3 %, 50.1 %, and 44.3 % respectively. Linkage analysis with the combined use of all four intragenic markers gave the informative result of 76.6% (383/500) in the Korean females. In prenatal diagnosis of a hemophilic family, a pregnancy woman was heterozygous with 3 intragenic (Intron 1, 13, and 22) and 1 extragenic St14 VNTR markers. She was considered to be a carrier, and carried a male fetus by AMXY PCR and chromosome analysis of amniocytes. Fetus did not have the mutant haplotype as his uncle, suggesting a normal male status. Our study demonstrates utility of two new intragenic markers in factor gene for the carrier detection and prenatal diagnosis of hemophilic families.

A genomic map of the candidate susceptibility loci of multiple sclerosis in N. Ireland based on the GAMES studies of European populations. *H.H. Abdeen¹, S. Heggarty², S. Hawkins³, M. Hutchinson⁴, C. Graham¹.* 1) Molecular Genetics Laboratory, Belfast City Hospital, Belfast, UK; 2) School of Pharmacy, Queens University Belfast, Belfast, UK; 3) Department of Neurology, Royal Victoria Hospital, Belfast, UK; 4) Department of Neurology, St Vincent's Hospital, Dublin, Eire.

Whole genome screening for association represents a powerful tool for identification of the susceptibility genes involved in complex genetic diseases. Assuming that at least the major susceptibility loci have arisen from common ancestors, then they may contain markers in linkage disequilibrium (LD) with the susceptibility variants. Depending on the extent of LD, such loci are expected to show some clustering of associated markers which are shared through multiple populations. We used the recently published data of case-control studies of the 16 European populations included in the Genetic Analysis of Multiple sclerosis in Europeans (GAMES) project, to construct a genomic map of the top microsatellite markers potentially associated with multiple sclerosis (MS). These have been selected from thousands of markers typed in separately pooled DNA from patients and controls of each population. The map was screened for loci showing clustering of associated markers through two or more populations. The strongest clustering was observed in the confirmed HLA locus on chromosome 6p21. Clustering was also observed in another 29 loci including 15 of the regions previously identified in the N. Irish GAMES study and 14 of other populations. These genomic regions contain genes with crucial functions in the nervous system and immunoregulatory genes, representing typical candidates for MS. To date, the top 11 loci have been further investigated by individual typing of several microsatellite markers each, in a N. Irish cohort of 200 cases and 200 controls. Association was confirmed for five of the loci reported in the N. Irish GAMES study (chromosomes 2p13, 4p16, 11q23, 19p13 and 19q13) and two of other populations (chromosomes 7q21 and 10p12). This refined screen of the human genome should provide a clearer image of the major susceptibility loci which may help the identification of the candidate genes involved in MS.

Fine mapping of the 5p13 locus linked to schizophrenia and schizotypal personality disorder in a Puerto Rican family. *I.N. Bespalova¹, G.W. Angelo¹, C.J. Smith¹, M. Durner³, L.J. Siever¹, J. Carrion-Baralt¹, J.D. Buxbaum^{1,2}, J.M. Silverman¹.* 1) Dept Psychiatry, Mount Sinai School of Medicine, New York, NY; 2) Dept Neurobiology, Mount Sinai School of Medicine, New York, NY; 3) Dept Biostatistics, Columbia University, New York, NY.

A locus involved in schizophrenia and related disorders has previously been mapped to chromosome 5p13 in a large Puerto-Rican family (Silverman et al., 1996). The family is from an isolated and genetically homogeneous population with a high frequency of relatively rare autosomal recessive diseases (Gershoni-Baruch et al., 1994; Anikster et al., 2001). The maximum two-point LOD score of 3.72 was obtained for marker D5S111, and rose to 4.37 by multipoint analysis, assuming autosomal dominant inheritance with 90% penetrance. Additional genotyping and haplotype analysis in this family placed the locus within a 4.8 cM genetic interval between markers D5S1993 and D5S631 (Durner et al., in prep). In this study, we focused on a branch of Family 17 containing two affected key recombinants flanking the critical interval on chromosome 5p13. Haplotype analysis revealed maternal allele sharing in a nuclear family with twelve siblings, three of whom are affected with schizophrenia and two with schizotypal personality disorder. We have genotyped five additional published microsatellite markers and 37 suspected densely spaced polymorphic markers across the genetic interval. We precisely mapped recombination breakpoints in two affected individuals, refined the boundaries and narrowed the minimal critical region (MCI) to 2.8 Mb. Since G-protein coupled receptors are plausible candidate genes for schizophrenia, and one of them is in the minimal critical region, SALPR, we analyzed this gene for mutations by sequencing the coding and the putative promoter regions in three siblings affected by schizophrenia. Although several new polymorphisms were detected, our results exclude SALPR as the gene for schizophrenia and related disorders in this Puerto Rican family.

Genome scan of working memory component phenotypes of dyslexia. Z. Brkanac^{1,2}, N.H. Chapman¹, R.P. Igo¹, J.B. Thomson¹, M. Matsushita¹, T. Holzman¹, V.W. Berninger¹, E.M. Wijsman¹, W.H. Raskind^{1,2}. 1) University of Washington, Seattle, WA; 2) VISN 20 MIRECC, VA PSHCS; Seattle, WA.

Dyslexia is a common disorder with complex genetics, as phenotypes do not show Mendelian patterns of inheritance. To reduce heterogeneity and increase the chances for linkage detection, we are using extensive phenotypic evaluations coupled with univariate component-based linkage analyses. We have previously shown through family aggregation and segregation analyses that working memory phenotypes have a genetic basis in our study families. We have assessed working memory with the Digit Span (DS) subtest of the WAIS-R and WISC-III. Phonological working memory was assessed with the Non Word Memory (NWM) task of the Comprehensive Test of Phonological Awareness. Families were collected through probands who had a Verbal IQ (VIQ)>90 and both performance below the population mean and discrepancy of 1 SD between VIQ and performance on at least one of ten dyslexia measures.

A genome scan with an average 10 cM resolution was performed on 438 people from 51 families selected on the basis of power to detect linkage for NWM. Multipoint Variance Component (VC) and Markov-chain Monte Carlo (MCMC) multipoint joint linkage and segregation analysis were performed. For NWM we observed numerous regions where VC dominance lod scores exceeded 1.5 and MCMC intensity ratios (IR) exceeded 5.0, well above the background level for the genome scan. VC-lod scores >2.0 that were supported by IR scores >5 were observed on chromosomes 2q, 4p, 6q, 17q, and 22q. VC-lod scores >1.5 were observed for DS on chromosomes 4q, 13p and 22q. MCMC IR results >5 for DS were detected on chromosomes 5q and 14q. Analysis of NWM adjusted for DS reduced the number of regions with VC-lod >1.5 and revealed an additional location on chromosome 7q with VC lod >2.0 and MCMC IR>5.

This study has identified novel loci potentially linked to NWM in families with dyslexia. DS has not shown strong evidence for linkage. Analysis of additional families will be needed to further refine these findings.

Sibpair linkage analyses using SNP genotypes as covariant suggests that two candidate genes 11 cM apart on chromosome 3 may independently contribute to prostate cancer risk. *Y. Ding, G. Larson, T.G. Krontiris, The ECOG E1Y97 Study Group.* Beckman Res Institute, City of Hope, Duarte CA.

We conducted single point linkage analysis of over 80 candidate genes in 402 brothers affected with prostate cancer from 201 families. Markers representing two adjacent candidate genes on chromosome 3p, *CDC25A* and *FHIT*, demonstrated suggestive evidence for linkage with identity by descent (IBD) allele-sharing statistics. Fine-structure multipoint linkage analyses were performed using LODPAL (S.A.G.E.) and MERLIN. The strongest evidence of linkage was detected for D3S1234 (located in intron 5 of *FHIT*) at 81.23 cM (maximum LOD score = 3.15, $p = 0.00007$) using LODPAL, and for both *CDC25a2* (15 kb downstream of *CDC25A*) at 70.55 cM ($NPL_{all} = 1.90$, $p = 0.03$) and D3S1234 ($NPL_{all} = 1.84$, $P = 0.03$) using MERLIN. For a subset of 38 families in which three or more affected brothers were reported, LODPAL generated a maximum LOD of 3.83 ($p = 0.00001$) at D3S1234 and a secondary peak of 2.19 at *CDC25a2*), while MERLIN produced a maximum NPL_{all} of 2.94 ($p = 0.002$) at *CDC25a2* and a smaller peak of 2.38 ($p = 0.009$) at D3S1234. We then genotyped 16 SNPs covering a 381 kb region surrounding D3S1234 and 5 SNPs spanning 148 kb region surrounding *CDC25A* on one case from each family. Using LODPAL with one-parameter model incorporating individual SNPs as covariate, we evaluated each SNP for their genotype correlation with excessive IBD sharing in all families. We found one SNP from each region with significantly increased maximum LOD scores of 5.02 and 4.72 at D3S1234 ($\alpha = 100$) and *CDC25a2* ($\alpha = 7$), respectively. Permutation tests of random SNP genotype designation to each family assuming the same genotype frequency, missing data, and value of α demonstrated a p value of ~ 0.01 for the associated SNP at D3S1234 and $p < 0.001$ for the SNP at *CDC25a2* to generate maximum LOD exceeding observed ones. These results suggest that both candidate genes *CDC25A* and *FHIT* may independently be involved in prostate cancer risk. They also demonstrate potential advantages using SNP genotypes as covariate to reduce heterogeneity and to pinpoint disease locus in the absence of unaffected controls.

Childhood cortical bone mass linked to chromosome 3p. *D.L. Duren¹, J. Blangero², T. Dyer², S.A. Cole², A.F. Roche¹, R.M. Siervogel¹, B. Towne¹.* 1) Wright State University School of Medicine, Dayton, OH; 2) Southwest Foundation for Biomedical Research, San Antonio, TX.

Genetic influences on bone mass are known almost entirely from studies of adults. Despite growing awareness regarding the importance of bone mineral accrual during childhood to later bone health, few studies have examined the genetic underpinnings of childhood bone mass. In this study, we examined radiographic cortical thickness of the second metacarpal in 600 participants from the Fels Longitudinal Study at chronological age 10 years. These individuals are from 144 nuclear and extended families. Medial and lateral cortical measurements and bone diameter, were taken perpendicular to the long axis of the bone. An initial set of 356 of these individuals have been genotyped for ~400 autosomal markers spaced approximately every 10 cM. A variance components-based linkage analysis method (SOLAR; Almasy and Blangero, 1998) was used to obtain multipoint LOD scores. Ten suggestive LOD scores (> 1.9) were found for the following traits: cortical index, total cortex, lateral cortex, medial cortex, and polar moment of inertia. More importantly, two significant LOD scores (> 3.0) were found for linkage of cortical thickness measures to markers on chromosome 3 a LOD of 3.29 for linkage of total cortex to a QTL on 3p at 24 cM, and a LOD of 4.13 for linkage of lateral cortex to a QTL on 3p at 26 cM (both located between markers D3S1304 and D3S1263). One potential positional candidate gene residing in this region is the OXTR (oxytocin receptor gene). Because the oxytocin receptor was recently found to be functional in both osteoblasts and osteoclasts, and has binding affinity to both oxytocin and estrogen, OXTR is a plausible candidate gene for childhood bone accrual. These results also suggest the existence of genes on chromosomes 2, 3, 4, 10, and 12 that influence measures of bone mass at age 10. Future work will seek to identify other specific genes that influence the accrual of bone mass at different stages of childhood development. Supported by NIH grants HD36342, HD12252, and MH59490.

Association and linkage analysis of the dyslexia susceptibility locus (DYX3) on chr 2p15-p16. *T. Fagerheim^{1,2}, E. McAuley², F-E. Tonnessen³, S.D. Smith⁴, R.K. Olson⁵, B.F. Pennington⁶, J.C. DeFries⁵, A.P. Monaco².* 1) Dept Medical Genetics, University of Tromsø, Tromsø, Norway; 2) Wellcome Trust Centre for Human Genetics, University of Oxford, UK; 3) University College of Stavanger, Centre for reading research, Stavanger, Norway; 4) Center for Human Genetics, University of Nebraska medical Center, Omaha, Nebraska; 5) Institute for Behavioral Genetics, University of Colorado, Boulder, Colorado; 6) University of Denver, Colorado.

Approximately 3-10% of people have specific difficulties in reading, despite normal intelligence, adequate education and social environment. Developmental dyslexia is viewed as a biologically complex behavioural disorder with genetic factors playing a significant role in predisposition towards the phenotype. We have previously mapped a susceptibility gene for dyslexia to 2p15-p16 (DYX3 locus) and the location has been replicated by independent studies in other populations (Canadian, UK, and US). We initiated SNP-based association screening of genes in the DYX3 locus and extended the analysis to genes surrounding the critical region. SNPs were selected based on LD information extracted from the HapMap project. 60 SNPs targeting 18 genes covering a 16.5 Mb region were genotyped in a sample of 120 twin-based families from Colorado. The most interesting association ($P = 0.006$) was found with a SNP within the NRXN1 gene for two reading related measures. In addition to association analysis, we decided to do a reanalysis of the DYX3 region from a previous genome-wide scan of the US families (120 original families) using the latest updated genetic map, new phenotypic data and including SNP data from the region. Multipoint Haseman-Elston linkage analysis gave the highest LOD score of 2.17 for reading related scores, and multipoint Variance component linkage analysis gave a maximum LOD score of 2.20 for an orthographic test. The results provided a refined candidate region. Additional SNPs will be selected for association and linkage analysis and additional sets of UK families and twin-based US families will be analysed for replication of any positive associations.

Genomic regions linked to fat storage in infants. *D. Fradin*¹, *S. Heath*², *M. Lathrop*², *P. Bougneres*¹. 1) Dpt of Pediatric Endocrinology & U561 INSERM, Hôpital Saint Vincent de Paul Paris, France; 2) National Center of Genotyping, Evry, France.

Fat stores deposited in response to abundant caloric supply are necessary to sustain the large needs for brain development in humans when infants face fasting periods. The ability for fat storage is thus likely to be an important physiological trait of human fitness. We used a sib-pair approach to characterize the genetic factors influencing fat deposition during the first two years of life. In a sample of 220 French families, we performed a genome wide linkage scan with 400 microsatellite markers to identify genomic regions that contain quantitative trait loci (QTLs) influencing body mass index (BMI), a quantitative measurement of fatness, at 2 years of age. After adjustment for sex, we found a strong linkage of the 8p12 region (LOD=2.56, $p=3.10^{-4}$) with infants BMI. The 7q31 region was also linked to BMI (LOD=1.11, $p=0.012$), as well as six other regions on chromosomes 15 (15q13.3 : D15S994, LOD=1.1, $p=0.012$), 10 (10q22-23 : D10S185, LOD=1.01, $p=0.002$), 5 (5p14.1 : D5S419, LOD=1.07, $p=0.013$ et 5q32 : D5S436, LOD=1.49, $p=0.004$), 8 (8q12.1 : D8S285, LOD=1.65, $p=0.003$ et D8S260, LOD=1.02, $p=0.02$) and 11 (11q22.1 : D11S4175, LOD=1.05, $p=0.014$ et D11S898, LOD=1.19, $p=0.01$). It is interesting that genome scans performed in adults have also identified the linkage of the 8p12, 7q31, 15q13.3 and 10q22-23 regions to obesity. No other regions showing linkage to infant BMI have been identified in adult studies of BMI. Our results suggest that the genetic factors influencing the variability of fat deposition in humans are not likely to be similar in infancy and adulthood ; however, some of them may be common to these two periods of life.

Comprehensive analysis of common variation in sex steroid pathway genes for a contribution to risk of SLE. *R. Graham*¹, *P. de Bakker*¹, *N. Burt*¹, *W. Ortmann*², *P. Rodine*², *B. Henderson*³, *D. Altshuler*¹, *T. Behrens*². 1) Molecular Biology, Harvard Medical School, Massachusetts General Hospital, Boston, MA; 2) Rheumatology and Autoimmune Diseases, School of Medicine, University of Minnesota, Minneapolis, MN; 3) Preventative Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA.

Autoimmune diseases typically exhibit a strong gender bias. In Systemic Lupus Erythematosus (SLE), the gender bias is among the most pronounced (female:male ratio, 9:1) and female gender remains the strongest known risk factor. Several lines of evidence indicate a direct role for sex steroids in modulating SLE incidence and severity, including: i) correlation of disease incidence with sex steroid hormone levels ii) direct modulation of sex steroid levels in murine models of lupus iii) efficacious treatment of model organisms and humans with anti-estrogens and androgens. Our goal is to test the hypothesis that inherited variation in the sex steroid hormone pathway influences individual risk of SLE. As part of an ongoing collaboration by Broad Institute of Harvard and MIT and the Multiethnic Cohort Study, genetic variation in 35 genes central to the sex steroid hormone pathway has been extremely well characterized. Coding regions have been resequenced in 192 individuals, and a total of 3,000 SNPs spanning 3Mb of DNA (>1 SNP per kb) have been genotyped in >2,000 chromosomes of diverse ancestry to define patterns of linkage disequilibrium. From this unusually rich dataset an efficient and informative set of SNPs (tagSNPs) have been selected to capture the vast majority of variants. We have identified and are completing genotyping of the first 750 tagSNPs from 35 genes in 215 SLE families (>1500 chromosomes). We will present analysis of single variants and combinations of variants for association to risk of SLE.

Combinatorial mismatch scan for loci associated with dementia in the Amish. *D.W. Hahs¹, A. Crunk¹, P.C. Gaskell², W.K. Scott², K.A. Welsh-Bohmer², S.R. Johnson², C.E. Jackson³, M.A. Pericak-Vance², J.L. Haines¹.* 1) Center for Human Genetics Research, Vanderbilt University, Nashville, TN; 2) Center for Human Genetics, Duke University, Durham, NC; 3) Scott & White, Temple, TX.

The Amish located in Indiana and Ohio are relatively isolated populations that may have increased power to detect disease susceptibility genes. Combinatorial mismatch scanning (CMS) (Heath et al., Hum Hered 2001;52:183) was developed to assess IBD sharing in distantly related individuals from such populations where standard linkage and association analyses are difficult to implement. Individuals used in this comparison were derived from six pedigrees with a total of 131 tested and genotyped individuals. Affected individuals were ascertained using the Modified Mini-Mental State (3MS) Examination and a score <87 was considered suspicious for cognitive impairment. 13 dementia cases and 13 normal controls were identified who were not related back to the grandparental generation. Comparisons were done using two Fishers exact tests, one for excess in IBD allele frequency and the other for excess in IBD genotype frequency at 417 microsatellite markers. Using the allele frequencies, 31 markers (7%) were associated with dementia at a nominal level of statistical significance ($p < 0.05$); the most significant ($p < 0.005$) were D3S1262, D5S211, D8S1477, and D19S1156. Using the genotype frequencies, 24 markers (6%) were statistically significant ($p < 0.05$); the most significant ($p < 0.005$) markers were D5S1347, D5S1480, and D5S211, spanning 36 cM. The only marker significant at the $p < 0.005$ level using both tests was D5S211. Three of the four markers with $p < 0.005$ on the allelic test were also significantly associated using the genotype test (for D19S1156, the genotype test p-value was 0.07). For nine markers p values < 0.05 were obtained for both tests: D3S1262, D4S1625, D5S211, D6S1031, D8S1477, D8S272, D10S1221, D17S921, and D18S481. Two-point lod score analysis detected a signal (lod = 2.155) at D4S1548, 8 cM from D4S1625 and another signal (lod = 2.188) at D5S1725 located 6 cM from D5S1347. The convergence of these two methods suggests that loci on chromosomes 4 and 5 may be involved in dementia.

Combined analysis of four genome scans indicates linkage with lipid parameters on chromosomes 2, 12 and 19.

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Elevated lipid levels in plasma are key risk factors for cardiovascular diseases. To map quantitative trait loci for lipid levels, genome-wide scans were performed in Dutch, Swedish and Australian twins. A new statistical procedure was devised to simultaneously analyse data from multiple genome scans. The study focussed on plasma levels of LDL-cholesterol and its protein constituent apolipoprotein B and on HDL-cholesterol and its main protein constituent apolipoprotein AI. Genome-wide scans with an average marker spacing of 5-10 cM were performed in 1388 dizygotic twins (694 pairs) from The Netherlands (200 pairs), Sweden (44 pairs) and Australia (450 pairs). For the simultaneous analysis, merlin-regress was used to estimate QTL heritabilities and standard errors for each of the populations separately. These estimates were then combined into a single heritability estimate by calculating the mean of population-specific heritabilities weighted according to the inverse of the squared standard error. Importantly, the analysis allows for heterogeneity between populations. LOD-scores higher than 2 were observed for HDL-C on chromosome 12q at 93 cM (LOD=2.5) and for LDL-C on 19q at 70 cM (LOD=2.4). On 2q at 186 cM, a LOD-score of 1.9 was observed for both LDL-C and ApoB levels. The contribution to the linkage signals was investigated for 19 SNPs in positional candidate genes using combined/linkage association analysis.

Genetic Association of TAS2R16 Taste Receptor on Chromosome 7 with Alcohol Dependence. A. Hinrichs¹, J.C. Wang¹, J.M. Kwon², S. Bertelsen¹, D. Dick¹, J. Budde¹, R. Allen¹, H. Stock¹, R. Crowe³, V. Hesselbrock⁴, M. Schuckit⁵, H. Begleiter⁶, B. Porjesz⁶, H. Edenberg⁷, T. Reich¹, L.J. Bierut¹, A.M. Goate¹. 1) Dept Psychiatry, Washington Univ Sch Med, St Louis, MO; 2) Depts of Neurology and Pediatrics, University of Rochester Medical Center, Rochester, NY; 3) University of Iowa School of Medicine, Iowa City, IA; 4) University of Connecticut School of Medicine, Farmington, CT; 5) UCSD School of Medicine, La Jolla, CA; 6) SUNY Health Science Center at Brooklyn, Brooklyn, NY; 7) Indiana University School of Medicine, Indianapolis, IN.

The Collaborative Study of the Genetics of Alcoholism (COGA) is a large multicenter study whose goal is to find genes that modify susceptibility to alcohol dependence and related phenotypes. A strong finding (LOD=2.9) on chromosome 7q is located at a cluster of bitter taste receptors (TAS2R). One member of this cluster, TAS2R16, is a receptor for bitter beta-glucopyranosides (such as salicin). We identified three polymorphic SNPs from the NCBI database including two non-synonymous coding SNPs, rs860170 and rs846664. The rs846664 SNP shows association using the TDT (with Transmit) for COGA (i.e. DSM-III-R and Feighner definite) and DSM-IV diagnoses of alcohol dependence ($p=0.01$ and 0.002). An additional set of trios (two parents and one COGA alcohol dependent child) unrelated to the linkage screening set was also genotyped and also showed association with the TDT ($p=0.03$). With the original sample and the additional trios we achieve a p -value of 0.002 with the COGA alcohol dependence diagnosis. We stratified the linkage sample into those families containing the mutation and those without. The pedigrees with the mutation have a sharing of 60.9% at the linkage peak on chromosome 7 for the COGA diagnosis. The families without the mutation have a sharing of 55.8% (overall sharing is 56.5%). It appears that although only 15% of the families have the mutation, those families contribute disproportionately to the linkage signal. Together these analyses provide strong evidence that variation in the TAS2R16 gene is associated with the risk for alcohol dependence.

Genome-Wide significant Non-MHC locus in Rheumatoid Arthritis families from Iceland. *A. Karason¹, K. Steinsson², V.B. Hauksson¹, E. Halapi¹, H. Jonsson¹, G. Jonsdottir¹, J.R. Gulcher¹, K. Stefansson¹, H. Hakonarson¹.* 1) deCODE Genetics, Reykjavik, Iceland; 2) University National Hospital, Reykjavik, Iceland.

Rheumatoid arthritis (RA) is a common complex inflammatory disease affecting approximately 1% of the population and is associated with reduced life expectancy. RA has a complex etiology with women being affected more frequently than men (sex ratio 3:1). Susceptibility to RA has long been associated with the Human Leukocyte Antigen (HLA) class. Specifically, the tightest association has been with DRB1*0401 and other class II HLA alleles, though it is speculated that the genetic contribution of the HLA alleles is less than 50%. RA may be triggered or exacerbated by several environmental factors such as smoking and it shows increasing frequency with increased hemispherical latitude. We performed a genome-wide scan with 1,200 microsatellite markers in 160 extended Icelandic families including 500 RA patients and over 900 of their first degree relatives. All study subjects signed an informed consent, underwent comprehensive phenotype studies to confirm the RA phenotype with respect to ACR criteria 1-7 and DRB1 status. Additional markers were typed at loci with lod scores of 2 or higher to increase the information content. Genome-wide linkage analysis demonstrated genome-wide significant (GWS) linkage on chr 3q with allele-sharing lod scores of 4.2. By typing 55 additional markers the lod on chr 3q rose from approximately 3 to 4.2 and the interval defining drop of one in lod (D3S1577 to D3S1271) narrowed to around 5 cM. We conclude that a GWS locus is present on chr 3q in patients with RA, a locus that corresponds in location to loci found in other autoimmune/inflammatory disorders. We anticipate that the gene we have mapped will significantly improve our understanding of the pathogenesis of RA and ultimately allow for new and more effective therapies to be developed.

Genetic linkage studies of stuttering in a large West African kindred. *B. Levis¹, D. Ricci¹, J. Lukong², D. Drayna¹.*
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Stuttering is a common speech disorder characterized by interruptions in the flow of speech, including prolongations of sounds, repetition of sounds or syllables and/or silent blockages. Although the fundamental cause of stuttering is unknown, both genetic and non-genetic factors are involved. Evidence for genetic factors of stuttering come primarily from twin studies, suggesting concordance rates of ~60% and ~20% in MZ and DZ twins, respectively, and a previously published linkage study of stuttering in North American families, identifying a locus chromosome 18. We are investigating the genetic causes of stuttering in a large, English-speaking West African family in which stuttering occurs as a trait with a simple inheritance pattern. This family contains 106 individuals in 5 generations, 45 of whom stutter as adults. We obtained DNA and speech samples from 44 members of this family and performed a complete genome wide scan using the Weber Marshfield panel 9, containing 394 microsatellite markers with an average spacing of 10 cM. Parametric multipoint linkage analyses under a model of autosomal dominant with reduced penetrance gave a maximum LOD score of 4.39 with markers on chromosome 1. To facilitate more precise localization of the gene causing stuttering in this family, we propose to search for a founder effect and Identity by Descent in apparently unrelated individuals in this population. To support this effort, we have obtained DNA and speech samples from 57 unrelated individuals who stutter and 50 normal controls from this population, and we are performing SNP genotyping for association studies at loci across the region showing linkage.

Dissection of BXSB lupus phenotype using mice congenic for chromosome 1 demonstrates that separate intervals direct different aspects of disease. *B.J. Morley¹, N.J. Rogers², S.J. Rose¹, J. Boyle³, S. Izui⁴, M.E.K. Haywood¹*. 1) Rheumatology Section, Eric Bywaters Centre, Faculty of Medicine, Imperial College, Hammersmith Campus, Du Cane Road, London, W12 0NN, United Kingdom; 2) Department of Immunology, Faculty of Medicine, Imperial College, Hammersmith Campus, Du Cane Road, London, W12 0NN, United Kingdom; 3) Histopathology Department, Hammersmith Hospital, Du Cane Road, London, W12 0NN, United Kingdom; 4) Department of Pathology, Centre Medical Universitaire, Geneva, Switzerland.

The BXSB mouse strain is an excellent model of proliferative glomerulonephritis seen in human Systemic Lupus Erythematosus (SLE) patients. Extensive linkage analysis has identified six non-MHC, autosomal loci (*Bxs1-6*) that contribute to SLE susceptibility in BXSB mice. In order to dissect the effects of the four chromosome 1 loci, we generated congenic lines on a C57BL/10.Y^{BXSB} (B10.*Yaa*) background.

Nephritis, qualitatively similar to BXSB, developed in three of these congenic strains. Early-onset, severe disease was observed in B10.*Yaa*.BXSB-*Bxs2/3* congenic mice and caused 50% mortality by 12 months. The delay in disease, compared to the BXSB parental strain, illustrated the necessity for multiple genetic interactions in the production of the full BXSB phenotype. Monocytosis, seen as a characteristic of BXSB was only present in the B10.*Yaa*.BXSB-*Bxs2/3* strain, and a recently developed sub-congenic has narrowed the genetic region responsible for this phenotype. In B10.*Yaa*.BXSB-*Bxs1/4* mice, disease progressed more slowly, resulting in 13% mortality at 12 months. B10.*Yaa*.BXSB-*Bxs1* mice, despite their genetic similarity to B10.*Yaa*.BXSB-*Bxs1/4* mice, developed only a low-grade glomerulonephritis with no detectable anti-dsDNA antibodies. Conversely, the *Bxs2* interval was linked to the production of anti-dsDNA Abs without concomitant glomerulonephritis. This panel of congenic mice provides an excellent resource in which to dissect the complexities of the genetic basis of lupus.

A study of linkage of anthropometric traits related to obesity in the Old-Order Amish. *G.J. Papanicolaou¹, P. Platte², C.M. Klein³, K.F. Doheny⁴, M.H. Roy-Gagnon¹, A.J. Stunkard⁵, C.A. Francomano⁶, A.F. Wilson¹.* 1) Genometrics Section, NIH/NHGRI/IDRB, Baltimore, MD; 2) Biological & Clinical Psychology, Univ. Wuerzburg, Germany; 3) Psychobiology & Psychosomatic Research, Univ. Trier, Germany; 4) CIDR, Johns Hopkins University School of Medicine, Baltimore, MD; 5) Dept. Psychiatry, Univ. Pennsylvania, Philadelphia, PA; 6) NIA, NIH, Baltimore, MD.

Today nearly two-thirds of the U.S. population are overweight and over thirty percent are obese. As part of an ongoing study of traits related to obesity in the Old-Order Amish, seven two- and three- generation families, totaling 157 individuals (with a mean sibship of 7.2), were assessed for 21 obesity related traits including fat mass, and height, weight, hip and waist measurements. Genotyping was performed at the Center for Inherited Disease Research (Baltimore, MD) with a modified Marshfield Genetics 8 marker set consisting of 384 short tandem repeat markers with an average distance of 9 cM. Pair-wise model-independent linkage analysis identified candidate regions ($p < 0.001$) for fat mass, height, hip circumference, lean body mass, maximum lifetime weight, waist circumference, weight, and waist to hip ratio. Markers on Chromosomes on 7q, 8q, and 11q map to a number of the traits (including: height, hip and waist circumference, study and maximum lifetime weight), suggesting possible pleiotropic effects or a common etiology for the phenotypes examined.

A QTL on 6p22 is showing significant association to dyslexia in two independent samples. *S. Paracchini*¹, *C. Francks*¹, *S.D. Smith*², *A.J. Richardson*³, *T.S. Scerri*¹, *L.R. Cardon*¹, *A.J. Marlow*¹, *I.L. MacPhie*¹, *J. Walter*³, *B.F. Pennington*⁴, *S.E. Fisher*¹, *R.K. Olson*⁵, *J.C. DeFries*⁵, *J.F. Stein*³, *A.P. Monaco*¹. 1) WTCHG, Univ Oxford, UK; 2) Dept of Pediatrics, Univ Nebraska, Omaha, NE; 3) Dept of Physiology, Univ Oxford, UK; 4) Dept of Psychology, Univ Denver, CO; 5) Institute for Behavioral Genetics, Univ Colorado, Boulder, CO.

Dyslexia is one of the most prevalent of childhood cognitive disorders, and is caused in large part by genetic factors. Linkage studies have identified several regions that may contain quantitative trait loci (QTL) for this disorder. The most consistently replicated linkage is on 6p21.3-23, which has been found in five independent samples, including our own (224 UK siblings). We have previously shown using multivariate linkage analysis that the QTL on 6p21.3-23 influences variability shared between reading-related measures, but not shared with measures of general intelligence (IQ). By controlling for IQ in linkage analysis we have now refined the mapping of the QTL to a 5.8 Mb interval. We then used association analysis in our sample of 224 UK siblings to identify an underlying QTL on 6p22. We detected significant association across a 77 kb region of strong intermarker linkage disequilibrium that spans the first four exons of *KIAA0319*, the entire *TTRAP* gene, and the first exon of *THEM2*. We replicated these associations in a second set of UK siblings, and in an independent sample of US twin-based sibships. One haplotype, with 12% frequency in both the UK and US samples, is significantly associated with poor performance on several reading-related measures ($P=0.005$ for irregular word reading, $P=0.00007$ for orthographic coding, $P=0.002$ for single word reading and $P=0.02$ for spelling in the UK sample, and $P=0.02$ for single word reading, spelling and phonemic awareness in the US sample). Mutation screening of the exons and predicted promoters of *TTRAP*, *KIAA0319* and *THEM2* in 32 probands did not reveal coding changes that have obvious disruptive effects on these genes or that tag the risk haplotype. Functional studies of these genes are now required to determine the mechanism of pathogenesis.

17q25 is a candidate susceptibility region for TS: a study of two independent family samples. *P. Paschou¹, Y. Feng², A.J. Pakstis¹, W.C. Speed¹, M.M.C. DeMille¹, J.R. Kidd¹, B. Jaghori², R. Kurlan³, D.L. Pauls⁴, P. Sandor^{2,5}, C.L. Barr^{2,6}, K.K. Kidd¹.* 1) Department of Genetics, Yale University School of Medicine, New Haven, CT, USA; 2) Toronto Western Research Institute, University Health Network, Toronto, ON, Canada; 3) Cognitive and Behavioral Neurology Unit, Department of Neurology, University of Rochester School of Medicine, Rochester, NY, USA; 4) Psychiatric and Neurodevelopmental Genetics Unit, Department of Psychiatry, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA, USA; 5) Department of Psychiatry, University of Toronto, Toronto, ON, Canada; 6) Department of Psychiatry, Brain and Behaviour Programme, The Hospital for SickChildren, Toronto, ON, Canada.

Tourette Syndrome (TS) is characterized by motor and phonic tics and high comorbidity rates with other neurobehavioural disorders. The complex genetic background of the disorder remains elusive. In this study we explored the possibility that 17q25 contributes to TS susceptibility. The initial scan of chromosome 17 performed on two large pedigrees provided a non-parametric lod score of 2.41 close to D17S928. Fine mapping with 17 additional microsatellite markers increased the peak to 2.61. The original families as well as two additional pedigrees were genotyped for 25 SNPs, focusing on three genes in the indicated region that could play a role in the development of TS. Multiple three-marker haplotypes spanning all three genes studied provided significant association results ($P < 0.001$). An independent sample of 96 small families was also studied. Three of the 25 SNPs were associated with TS. The transmission/disequilibrium test for a three-marker haplotype moving window again provided multiple positive results. The background linkage disequilibrium (LD) of the region was studied in eight European populations. The pairwise tests produced unexpectedly high LD values at the telomeric TBCD gene. In conclusion our findings warrant the further investigation of 17q25 as a candidate susceptibility region for TS. [This work has been supported by NINDS-R01-NS40024. P. Paschou is supported by a Tourette Syndrome Association Research Grant Award.].

Fine-mapping of an asthma susceptibility locus on chromosome 12q using a high-resolution ldTag SNP set. *B.A. Raby¹, R. Lazarus¹, E.K. Silverman¹, A. Montpetit², J.F. Olivier², T.J. Hudson², S.T. Weiss¹*. 1) Channing Laboratory, Brigham and Women's Hospital, Boston, MA; 2) McGill University and Genome Quebec Innovation Center, Montreal, QC.

Chr.12q is among the regions most commonly linked to asthma in both genome-wide and regional linkage studies. At least two distinct loci on 12q have been described: a 28.5cM region located between 48-76.5Mb from pter, and a more telomeric locus at 108-128Mb. We have previously demonstrated evidence for linkage with asthma to the proximal locus in 55 nuclear families ascertained through the Childhood Asthma Management Program (CAMP). To narrow this region more precisely, we are using a high-resolution SNP map across the 28.5Mb region that will be genotyped using the Illumina BeadArray platform in 400 asthmatic children and their parents. SNPs selected for genotyping represent a subset of 4025 SNP with genotype data generated by the international HapMap project on 30 Caucasian CEPH trios (Jan 2004 release). We applied an algorithm that selects an optimal subset of SNP for genotyping of minimal size to assure that all SNPs that will not be genotyped are in strong linkage disequilibrium (r^2 of at least 0.9) with those that are typed. 1611 ldTag SNPs were identified (representing 64% of all SNPs with minor allele frequencies of at least 0.05 and less than 5% missing genotype data). Median r^2 between these 1611 SNPs and the remaining unselected SNPs was 0.97. From this subset assays were designed for 1536 and typed on 400 nuclear families. The June 2004 HapMap data release included ~ 2000 more SNPs for this region. We evaluated the ability of the LD-tagging SNP set generated from the original data to tag these additional SNPs. For the 3192 polymorphic (minimum MAF 0.05) SNPs not typed, the median r^2 with the typed ldTag SNPs was 0.93, with less than 22.5% of untyped SNPs having r^2 of 0.5 or less. These preliminary analyses suggest that the combined use of HapMap data and an LD-tagging fine mapping approach will provide adequate genomic coverage to enable localization of genetic variants in complex traits. The pending results of the family-based association analysis in our asthma study will be presented.

Chromosomes 4q35 and 16q23 show evidence of linkage to autoimmunity loci in lupus pedigrees. *P.S. Ramos¹, J.A. Kelly², A.N. Leiran¹, C.M. Meyer¹, K.J. Espe¹, W.A. Ortmann¹, G.R. Bruner², J.M. Olson³, T.W. Behrens¹, J.B. Harley², K.L. Moser¹.* 1) University of Minnesota, Minneapolis, MN; 2) Oklahoma Medical Research Foundation, Oklahoma City, OK; 3) Case Western Reserve University, Cleveland, OH.

Numerous studies have suggested the existence of autoimmunity-predisposing genes. We have used autoimmunity traits as intermediate phenotypes for mapping genes in systemic lupus erythematosus (SLE) and other autoimmune diseases. We have defined autoimmunity traits based on lupus-related autoantibody profiles in 229 families multiplex for SLE. We measured frequencies, familial aggregation and correlations of autoantibodies directed against Ro/SSA, La/SSA, nRNP, Sm, dsDNA, rheumatoid factor, and antinuclear antibodies (ANAs) and antiphospholipid antibodies (aPL IgG and aPL IgM). We identified evidence of familial aggregation for anti-La, anti-nRNP, and anti-Sm ($p < 0.05$). We then performed linkage analysis using these traits in two independent pedigree collections (OMRF and MN). Non-parametric genome wide linkage analyses were performed using both the revised Haseman-Elston algorithm (SIBPAL) and an affected relative pairs approach (LODPAL) to identify chromosomal regions of increased allele sharing. We obtained significant evidence for linkage ($p < 0.000022$) on chromosome 16q23 with anti-nRNP as the trait in the OMRF collection, and suggestive evidence ($p < 0.00074$) in the same region with ANAs in the MN collection. In previous studies, significant evidence for linkage to type I diabetes has been reported to this same region. We also obtained suggestive evidence for linkage on 4q35 when using the presence of Ro and/or La autoantibodies as the trait with the OMRF collection. This region is of interest because it overlaps with the psoriasis PSORS3 locus, and both anti-Ro and anti-La are associated with cutaneous manifestation of SLE. Additional loci were identified using other autoantibody traits. These results show that using autoantibody traits as intermediate phenotypes provides evidence of the presence and locations of genes that are involved in the development of autoimmunity.

A novel locus on chromosome 2 for speed of phonological decoding. *W.H. Raskind^{1,2}, R.P. Igo³, N.H. Chapman³, J.B. Thomson⁴, M. Matsushita¹, Z. Brkanac^{2,6}, T. Holzman⁵, M. Brown¹, V.W. Berninger⁴, E.M. Wijsman^{1,3}.* 1) Departments of Medicine; 2) Psychiatry and Behavioral Sciences; 3) Biostatistics; 4) and Educational Psychology; 5) and the Locke Computer Center, University of Washington; 6) and VAPSHCS, Seattle, WA.

Dyslexia is a common developmental disorder manifested by unexpected difficulty in learning to read. Impaired phonological decoding (translation of written words into spoken words, without meaning cues) is thought to be the core deficit. We present a 10 cM genome scan of two continuous measures of phonological decoding ability: word attack (WA), and phonemic decoding efficiency (PDE). PDE measures both accuracy and speed of phonological decoding, whereas WA measures accuracy alone. Analyses were performed for PDE, WA, and PDE adjusted for WA using multipoint variance component linkage analysis (VC) and Markov chain Monte-Carlo (MCMC) multipoint joint linkage and segregation analysis methods, on 108 families containing 874 people in total. A subset of 51 families comprised of 438 sampled people was genotyped. The remaining families were used for parameter estimation. The MCMC results are reported as intensity ratios (IRs), which are estimates of the ratio of the posterior to prior probability of linkage.

The strongest signal was observed on chromosome 2 for PDE using both VC and MCMC methods (lod=2.65, IR=32.1). This signal was not seen for WA. For PDE adjusted for WA, the VC signal decreased but the MCMC signal was enhanced (lod=1.65, IR=74.2). Additional markers were genotyped in the region, and the lod score and IR for PDE increased to 3.0 and 59.5, respectively. Parametric analyses of PDE were also performed, using a model obtained by complex segregation analysis on 235 families. Maximum single-marker and multipoint lod scores were 1.84 and 2.89, respectively. In contrast, we found no evidence for linkage to regions on chromosomes 1, 2, 6, 11, or 18 that were previously implicated in phonological decoding. The consistency of results from three analytic approaches provides strong evidence for a locus on chromosome 2 that influences speed but not accuracy of phonological decoding.

Linkage Studies of Stuttering in Inbred Pakistani Families. *N. Riaz*¹, *S. Steinberg*³, *J. Ahmad*², *S. Riazuddin*², *A. Pluzhnikov*³, *N. Cox*³, *D. Drayna*¹. 1) NIDCD/NIH, Rockville, MD; 2) CEMB, University of Punjab, Lahore, Pakistan; 3) Department of Human Genetics, University of Chicago, Chicago, IL.

Stuttering is a communication disorder that affects speech fluency, and is diagnosed by the presence of syllable repetitions, syllable prolongations, and interruptions in the smooth flow of speech known as blocks. Many types of evidence suggest genetic factors contribute to stuttering. Because the genetics of stuttering is complex, we have sought specialized populations to give added power to linkage studies. One hundred consanguineous stuttering families were identified from the city of Lahore and areas around Lahore, Pakistan, where consanguineous matings are the norm. Blood and diagnostic speech samples were collected from 56 families where there were more than 3 affected individuals. Diagnosis was performed using Stuttering Severity Instrument, and quantitative measures of affection status were obtained. The male to female ratio in Pakistani stutterers is 4:1, similar to that observed in other populations. A genome wide linkage scan was done using affected individuals and their parents in 44 selected families, genotyped with the Marshfield Weber 9 Panel. Analysis was performed on 144 affecteds, 55 unaffecteds, and 27 population matched normal controls. PREST was used to identify pedigrees that required additional specification of inbreeding. Initial non-parametric analysis using Merlin gave evidence of linkage on chromosomes 1q, 11q, 12q and 18q. Additional genotyping was performed at these four linked loci to a level of 5 cM resolution, and 16 additional individuals within the same family set were included. Analysis of the enlarged data set provided consistent significant evidence for linkage on chromosome 12: the S_{homoz} scoring function in Allegro gave a LOD score of 4.68, and a LOD score of 3.29 was obtained using the S_{all} scoring function. These results suggest that a locus on chromosome 12q may contain a gene with a large effect in this population.

Analysis of Genetic Admixture and Asthma Related Phenotypes in Mexican American and Puerto Rican

Asthmatics. *K. Salari^{1,2}, S. Choudhry^{1,2}, H. Tang³, D. Lind¹, P. Avila¹, N. Coyle^{1,2}, N. Ung^{1,2}, S. Nazario⁴, J. Casal⁴, A. Torres⁴, A. Phong¹, I. Gomez⁴, E.J. Pérez-Stable¹, M.D. Shriver⁵, P.Y. Kwok¹, D. Sheppard^{1,2}, W. Rodriguez-Cintron⁴, N.J. Risch^{6,7}, E.G. Burchard^{1,2}, E. Ziv¹.* 1) University of California, San Francisco, San Francisco, CA; 2) Lung Biology Center, San Francisco General Hospital, San Francisco, CA; 3) Fred Hutchinson Cancer Research Center, Seattle, WA; 4) San Juan VAMC, University of Puerto Rico School of Medicine, San Juan, PR; 5) Department of Anthropology, Pennsylvania State University; 6) Department of Genetics, Stanford University School of Medicine, Stanford, CA; 7) Division of Research, Kaiser Permanente, Oakland, CA.

Genetic association studies in admixed populations may be biased if individual ancestry varies within the population and the phenotype of interest is associated with ancestry. However, recently admixed populations also offer potential benefits in association studies since markers informative for ancestry may be in linkage disequilibrium across large distances. In particular, the enhanced LD in admixed populations may be used to identify the alleles which underlie a difference in a phenotype between two ancestral populations. Asthma is known to have different prevalence and severity among ancestrally distinct populations. We investigated several asthma-related phenotypes in two ancestrally admixed populations: Mexican Americans and Puerto Ricans. We used ancestry informative markers to estimate the individual ancestry of 181 Mexican American asthmatics and 181 Puerto Rican asthmatics and tested whether individual ancestry is associated with any of these phenotypes independently of known environmental factors. We found a strong association between higher European ancestry and more severe asthma as measured by both forced expiratory volume at 1 second ($r=-0.21$, $p=0.005$) and by a clinical assessment of severity among Mexican Americans (OR: 1.37, $p=0.001$). Among Puerto Ricans we found a modest association between African ancestry and improved drug responsiveness. These results suggest that asthma severity, particularly among Mexican Americans, may be influenced by genetic factors differentiating Europeans and Native Americans.

Loci contributing to adult height and body mass index in African American families ascertained for type 2 diabetes. *M.M. Sale*^{1,2}, *B.I. Freedman*², *P.J. Hicks*^{1,3}, *A.H. Williams*⁴, *C.D. Langefeld*⁴, *C.J. Gallagher*^{1,3}, *D.W. Bowden*^{1,2,3}, *S.S. Rich*⁴. 1) Center for Human Genomics, Wake Forest University School of Medicine, Winston-Salem, NC; 2) Dept. Internal Medicine, Wake Forest University School of Medicine, Winston-Salem, NC; 3) Dept. Biochemistry, Wake Forest University School of Medicine, Winston-Salem, NC; 4) Dept. Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, NC.

Height and body mass index (BMI) have high heritability in most studies, and high BMI and reduced height are important risk factors for a number of diseases. We investigated these phenotypes in African American families originally ascertained for studies of linkage with type 2 diabetes. We conducted a genome wide scan in 218 families containing 580 individuals of African-American descent. Estimates of heritability and support for linkage were assessed by genetic variance component analyses using SOLAR software. The estimated heritabilities for height and BMI were 0.43 and 0.64 respectively. We have identified major loci contributing to variation in height on chromosomes 15 (LOD=3.19 at 35 cM), 3 (LOD=2.17 at 84 cM), 8 (LOD=2.34 at 135 cM) and 17 (LOD=2.04 at 110 cM). A broad region on chromosome 4 supported evidence of linkage to variation in BMI, with the highest LOD=2.99 at 168 cM. We report the results of genome-wide scans for height and BMI in African American families. All major regions of linkage appear to confirm the existence of quantitative trait loci previously identified by other studies, providing important replicative data to allow further resolution of linkage regions suitable for positional cloning these risk loci.

A genome-wide linkage scan for quantitative trait loci influencing the craniofacial complex. *R.J. Sherwood¹, D.L. Duren¹, J. Blangero², T. Dyer², S.A. Cole², R.M. Siervogel¹, B. Towne¹.* 1) Wright State University School of Medicine, Dayton, OH; 2) Southwest Foundation for Biomedical Research, San Antonio, TX.

Recent advances in developmental biology have led to the discovery of a number of genes and gene products important in the development of the skeleton and, specifically, the skull. However, as is the case with many systems, our primary understanding of the specific genes related to morphology comes from genetic disorders associated with craniofacial anomalies. In this study, we examined nine quantitative traits taken from lateral skull radiographs of 510 healthy participants from 90 families in the Fels Longitudinal Study. An initial set of 197 of these individuals have been genotyped for ~400 autosomal markers spaced approximately every 10 cM. A variance components-based linkage analysis method (SOLAR; Almasy and Blangero, 1998) was used to obtain multipoint LOD scores. Nine suggestive LOD scores (> 1.9) were found for the following craniofacial measures: posterior-anterior, basion-sella, sella-sphenoethmoidale, basion-nasion, sella-vertex. These suggestive linkages were to markers on chromosomes 3, 6, 7, 12, 13, and 22. Of these, one region is of particular interest. The QTL interval for Chromosome 13 is broad and contains two highly suggestive linkage peaks for the trait Sella-Sphenoethmoidale, one at 67 cM (LOD = 2.33) between markers D13S170 and D13S265, and the other at 108 cM (LOD = 2.57) between markers D13S1265 and D13S285. These regions covered contain genes of potential interest, most notably the ZIC2 and ZIC5 genes. Both ZIC2 and ZIC5 encode a member of the ZIC family of C2H2-type zinc finger proteins. Mutations in either gene have been shown to cause holoprosencephaly type 5, a major congenital malformation often characterized by severe craniofacial anomalies. Additionally, SPRY2, a fibroblast growth factor antagonist, is found near the 67cM peak. Several craniofacial anomalies have been associated with fibroblast growth factor mutations. Future work will seek to identify the specific genes that influence variation in the morphology of the craniofacial complex. Supported by NIH grants HD36342, HD12252, MH59490.

A GENOME SEARCH FOR GENETIC DETERMINANTS THAT INFLUENCE PLASMA FIBRINOGEN LEVELS. *J.M. Soria¹, L. Almasy², J.C. Souto¹, A. Buil¹, M. Lathrop³, J. Fontcuberta¹, J. Blangero².* 1) Dept Hematology, Hosp de Sant Pau, Barcelona, Spain; 2) Southwest Foundation for Biomedical Research, San Antonio, TX, USA; 3) Centre National de Genotypage, Evry, France.

It is widely accepted that fibrinogen levels are strongly, consistently, and independently related to cardiovascular risk. These levels are influenced by genetic and environmental factors. Among the genetic factors, only some polymorphisms in the fibrinogen genes have been reported, and they explain only a small proportion of the genetic variability. Consequently, the extent of the genetic contribution is largely unknown. To search for these genes, we conducted a genome-wide scan using 21 Spanish families from the Genetic Analysis of Idiopathic Thrombophilia (GAIT) Project. Two loci were detected, one on chromosome 12 and another on chromosome 14. There are no cardiovascular-related candidate genes on chromosome 14 which implies that this locus represents a novel cardiovascular risk factor. Importantly, the locus on chromosome 12 contains a candidate gene (TCF1) involved in the hepatocyte-specific transcription of the fibrinogen alpha and beta chain genes. Several polymorphisms in this gene showed significant association with fibrinogen levels, supporting the implication of the TCF1 gene in the determination of this phenotype. It is interesting that the chromosome 4 region, where the structural fibrinogen genes are located, showed weak evidence of linkage, indicating these genes play a minor role in the normal variation of fibrinogen levels. Our results provide strong evidence that two loci, one on chromosome 12 (most likely the TCF1 gene) and another on chromosome 14, are important determinants of plasma fibrinogen levels in Spanish families. These data should help to define the relationship between fibrinogen levels and the risk of cardiovascular disease.

Analysis of the Tasmanian multiple sclerosis genome wide scan haplotype dataset. *J. Stankovich¹, M. Bahlo¹, J.P. Rubio¹, T.J. Kilpatrick^{1,2}, C.R. Wilkinson¹, T.P. Speed¹, S.J. Foote¹.* 1) Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia; 2) Howard Florey Institute, University of Melbourne, Victoria, Australia.

Multiple sclerosis (MS) is a debilitating complex genetic disease with no cure and limited treatment options. Identification of genes involved in MS is a difficult task and has so far only shown replicated association with the HLA region. We have conducted a genome wide case control haplotype sharing study in Tasmanian MS sufferers. The Tasmanian MS haplotype sharing study consists of 340 case haplotypes and 210 control haplotypes. The haplotypes were generated through probabilistic haplotype reconstruction using a number of close relatives. The study design features two sets of independent controls: a family based control (non transmitted haplotypes) and a population based control. The study design allows the testing of negative control datasets where the family based controls are compared to the population based controls as a valuable background LD check and population stratification check.

The genome wide scan analysis and ongoing fine mapping has so far identified two, low risk, common haplotypes outside of the HLA region. We show how the study design, the ongoing fine mapping and peripheral analysis are giving us added confidence in our findings.

A Genomewide Screen of 165 Families for Adolescent Onset Idiopathic Generalized Epilepsy (IGE) Susceptibility

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Genetic factors play the major role in the etiology of idiopathic generalized epilepsy (IGE). But identifying the susceptibility loci for IGE is confounded by ambiguous phenotype definition and heterogeneity. Recent findings (Durner M 2001, Winawer MR 2003) have suggested that the clinical definition of an IGE syndrome may not reflect the underlying genotype(s) as the components of the syndrome definitions overlap. Linkage analysis on the basis of seizure phenotype rather than IGE syndrome may therefore represent a more accurate phenotype definition for gene identification. Our previous genome scan for IGE susceptibility loci included 91 families and showed 1) strong evidence for linkage of IGE on chr. 18 (LOD=5.2 at D18S474) (Durner et al., 2001); 2) linkage of absence seizures to two markers on chr. 5; 3) linkage of tonic-clonic seizures to a marker on chr. 8 (Durner M, 1999); 4) replicated evidence of linkage of myoclonic seizures to chr. 6 (Greenberg et al, 2000). In this follow up study, we have completed whole-genome genotyping, data cleaning, and error checking for 74 multiplex IGE families. Analysis of this large, independent data set will provide a critical test of replication for the initial findings since the sample has been ascertained, diagnosed, and analyzed under conditions that closely match those of the previous study. We will present a complete analysis of the 74 families, and the combined 165 IGE families, exploring clinical phenotype, and more specifically examining seizure type as the critical phenotype for gene identification.

Linkage disequilibrium mapping for body mass index on chromosome 7q31-7q34 implicates multiple genes and sex-specific effects: The NHLBI Family Heart Study. *J.B. Wilk^{1,2}, Y. Jiang¹, S. Williamson¹, R. Prakash¹, A.L. DeStefano^{1,3}, R.C. Ellison², I.B. Borecki⁴, M.A. Province⁴, R.H. Myers^{1,2}.* 1) Department of Neurology, Boston University School of Medicine, Boston, MA; 2) The Section of Preventive Medicine and Epidemiology, Department of Medicine, Boston University School of Medicine, Boston, MA; 3) Biostatistics Department, Boston University School of Public Health, Boston, MA; 4) Division of Biostatistics, Washington University School of Medicine, Saint Louis, MO.

The NHLBI Family Heart Study (FHS) genome-wide scan identified a region of chromosome 7q with a LOD score of 4.9 for body mass index (BMI). We report the results of linkage and association to BMI in 90 FHS pedigrees using 418 SNPs and 7 microsatellites genotyped on 7q31-34. BMI was adjusted for age and study center, by sex, and linkage and association analyses were performed in the full sample and sex-specific subsets. Variance component linkage analysis using microsatellite data produced a LOD score of 16.4 positioned at D7S1804 (137 cM, 127 Mb Celera map). Linkage analysis using only SNPs produced a LOD of 16 at the same position, and sex-specific analysis produced LOD scores of 6.1 in females and 2.3 in males. Single SNP and haplotype association was evaluated using family-based association tests in the FBAT program, testing for association in the presence of linkage. Haplotype association results in the full sample implicated the testis development gene (NYD-SP18) at 123.15 Mb ($p=0.002$ for recessive model) and the exocyst complex component SEC8, at 128 Mb ($p=0.0008$ for dominant model), and these haplotypes were also the best results in females. In males, a haplotype in maltase-glucoamylase (MGAM) at 136.4 Mb had the best association ($p=0.002$ for additive model). Haplotypes in CALD1 and CNOT4 also produced results warranting further investigation. These data support the hypothesis that there are multiple genes on 7q31-34 influencing BMI and these genes may have sex-specific effects.

Replication study supports evidence for linkage to 9p24 in Obsessive-Compulsive Disorder. *V.L. Willour¹, Y.Y. Shugart¹, J. Samuels¹, M. Grados^{1,2}, B. Cullen¹, O.J. Bienvenu¹, Y. Wang¹, K.-Y. Liang¹, D. Valle¹, R. Hoehn-Saric¹, M. Riddle¹, G. Nestadt¹.* 1) Johns Hopkins University, Baltimore, MD; 2) Kennedy Krieger Institute, Baltimore, MD.

Obsessive-compulsive disorder (OCD) is a severe psychiatric illness that is characterized by intrusive and senseless thoughts and impulses (obsessions) and repetitive behaviors (compulsions). Family, twin, and segregation studies support the presence of both genetic and environmental susceptibility factors. The only published genome scan for OCD identified a candidate region on 9p24 at marker D9S288 that met criteria for suggestive significance (Hanna et al. 2002). In an attempt to replicate this finding, we genotyped 50 OCD pedigrees using microsatellite markers spanning the 9p24 candidate region and analyzed the data using parametric and nonparametric linkage analyses under both a narrow phenotype model (DSM IV OCD definite; 41 affected sib pairs) and a broad phenotype model (DSM IV OCD definite and probable; 50 affected sib pairs). Similar to what was described by Hanna et al. (2002), our strongest findings came with the dominant parameters and the narrow phenotype model: the parametric signal peaked at marker D9S1792 with an HLOD of 2.26 ($= 0.59$), and the nonparametric linkage signal peaked at marker D9S1813 with an NPL of 2.52 ($p=0.006$). These findings are striking in that D9S1813 and D9S1792 lie within 0.5 cM (<350 kb) of the original 9p24 linkage signal at D9S288. Furthermore, pedigree-based association analyses also implicated the 9p24 candidate region by identifying two markers (D9S288 and GATA62F03) with modest evidence ($p= 0.046$ and 0.02 , respectively) for association.

Mapping susceptibility loci for idiopathic scoliosis. *C.A. Wise¹, J.A. Herring¹, X.C. Gao¹, N.M. Cain¹, S.L. Swaney¹, S. Bashiardes², R. Veile², A.M. Bowcock², M. Lovett².* 1) Seay Research Ctr, Texas Scottish Rite Hosp, Dallas, TX; 2) Genetics Department, Washington University School of Medicine, St. Louis, MO.

Scoliosis is an isolated, lateral curvature of the spine with rotation that can require surgical correction. The most common form of scoliosis is idiopathic (IS) with onset during adolescence. It has a prevalence of 1-3% in school age children and a well-described 5:1 female:male ratio in children requiring treatment. The underlying causes of IS have remained elusive, although there is some suggestion of a neurological pathogenesis. IS inheritance is complex, and a number of linked loci have been reported. We tested replication of these linkages in set of mostly nuclear IS families. A minimum Cobb angle of 15 degrees with no other associated disorders was required to be considered affected. Sixty-one affected and seventy-two unaffected individuals representing 28 families were included in the analysis. Forty-four polymorphic microsatellites from 12 chromosomal loci were genotyped and searched for evidence of linkage using Genehunter. In the combined families small positive nonparametric scores were obtained for several loci, with strongest results produced for a region of chromosome 8 (NPL = 1.52, $p=.058$ at D8S1136). This was the only region producing a positive lod score when a dominant inheritance model was applied. These scores increased when combined with data from genome wide scans in two extended families (LOD = 3.12, NPL of 2.11 ($p=0.016$) at marker D8S1136). This region encodes the SNTG1 gene that we recently reported is disrupted in an affected family with (inv(8)(p23q11.21), providing independent evidence that the chromosome 8 locus contributes to IS susceptibility. Screens of SNTG1 coding regions revealed no obvious, co-segregating mutations in the family set. To test association between common variants in SNTG1 and IS we have initiated genotyping single nucleotide polymorphisms (SNPs) in a new collection of over 90 families. SNTG1 encodes a neuron-specific member of the syntrophin family and is a plausible candidate gene for IS susceptibility contributed by the chromosome 8 region.

Pleiotropic manifestation of lipid pathway genes affecting blood pressure, obesity, glucose and insulin resistance: evidence for metabolic syndrome genes in the MACAD Study. X. Guo^{1,2}, M.O. Goodarzi^{1,2}, M.J. Quinones², J. Cui¹, S. Cheng³, R. Hughes³, J. Wang³, K.D. Taylor^{1,2}, H. Yang^{1,2}, W. Hsueh², J.I. Rotter^{1,2}. 1) Cedars-Sinai, LA; 2) UCLA, LA; 3) Roche Molecular Systems, Inc., Alameda, CA.

The metabolic syndrome (MS), characterized by the clustering of central obesity, hypertension, higher triglyceride levels, lower HDL-cholesterol concentrations, and impaired fasting glucose, is a multiplex risk factor for cardiovascular disease and type 2 diabetes. A number of genes have been reported to be associated with lipoprotein metabolism, including Lp(a), ApoA4, ApoB, ApoC3, ADRB3, CETP, LIPH, LDLR, PON1, PON2, and PPARG. We evaluated the association of these genes with other components of the MS. 656 individuals from 102 Mexican American (MA) families recruited through a coronary artery disease proband in the MA Coronary Artery Disease (MACAD) project were genotyped for 24 polymorphisms in the above 11 genes by a PCR- and immobilized probe-based multi-locus assay. We evaluated the association between each polymorphic marker and the other MS-related traits via generalized estimating equation (GEE) methods. The T allele of T3206G in ApoC3 was found to be associated with obesity: BMI ($p=.013$), elevated fasting glucose ($p=.014$), and insulin resistance: fasting insulin ($p=.009$), HOMA ($p=.021$), GINF ($p=.017$), SI ($p=.014$) when adjusting for age and sex; but the association become non-significant after further adjusting for BMI. The C allele of C(-480)T in the LIPH gene was associated with obesity: trunk fat ($p=.006$), %bodyfat ($p=.007$), and insulin resistance: fasting insulin ($p=.021$), HOMA ($p=.006$) after adjusting for age, sex, and BMI. E360H in ApoA4, T71I in ApoB, C(-641)A and C1100T in ApoC3, W64R in ADRB3, S311C in PON2, and P12A in PPARG showed associations with insulin resistance ($p: .0008-.0375$), while C93T in the Lp(a) gene showed association with diastolic blood pressure ($p=.016$). In conclusion, lipid metabolism genes, especially ApoC3 and LIPH, may be genetic markers for the metabolic syndrome. While obesity may affect the expression of ApoC3, the LIPH effect is apparently independent of obesity.

Transmission Disequilibrium Tests For Quantitative Traits Via Semi-Parametric Regression. *S. Ghosh¹, C. Mukherjee¹, H. Begleiter², L. Bierut³*. 1) Applied Statistics Unit, Indian Statistical Inst, Kolkata, India; 2) Neurodynamics Laboratory, State University of New York at Brooklyn, NY; 3) Department of Psychiatry, Washington University School of Medicine, St. Louis, MO.

The classical transmission disequilibrium test (TDT) proposed by Spielman et al. (1993) has proved to be a powerful method for simultaneous detection of both linkage and association for binary traits. The paradigm of association in the context of quantitative traits is not very obvious and methods (Allison 1997, George et al. 1999, Abecassis et al. 2000, Monks and Kaplan 2000, Lange et al. 2002) have generally considered the intuitive concept of differences in allelic transmissions between offspring having high quantitative trait values and those having low values as evidence of linkage disequilibrium for quantitative traits. However, many of these tests use selected sampling resulting in loss of information and power or are not robust to violations in distributional assumptions of the quantitative trait. We propose a logistic link function between a marker allele transmitted by a heterozygous parent and the quantitative trait value of an offspring. This method can be shown to be statistically equivalent to the classical TDT. For multiple sibs, we develop a non-parametric regression based on kernel smoothing of the proportion of one marker allele transmitted by heterozygous parents to all sibs in a family on the mean of the quantitative trait values of the sibs. Monte-Carlo simulations are included to assess the powers of our methods. We also illustrate an application of our method using data on a quantitative phenotype defined as the number of externalizing symptoms and the ADH3 marker in the alcohol-dehydrogenase gene cluster on Chromosome 4 in the ongoing Collaborative Study On The Genetics Of Alcoholism project.

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Genetic dissection of Stress Vulnerability and Antidepressant Response in a Mouse Model. *H.K. Gershenfeld, X. Liu, T. Amstein, D. Stancliffe.* Dept Psychiatry, UT Southwestern, Dallas, TX.

The tail suspension test (TST) is a simple validated screening test for the behavioral effects of antidepressants in mice. Two parental inbred strains, NMRI and 129S6, markedly differ in their responses to stressful situations measured by duration of immobility in the TST and the effects of imipramine (a tricyclic antidepressant) in reducing immobility during a 6-min test. An F2 intercross was derived from NMRI and 129S6 inbred strains (N=747 males). Both parental strains and F2 mice underwent a standardized 6-min protocol of automated TST with 2 sessions: (1) baseline and (2) imipramine (30 mg/kg, i.p.) TST. A whole genome scan of the F2 intercross with 125 markers mapped two significant basal TST quantitative trait loci (QTL) on Mus chromosomes 5 (peak 47.6 cM, Lod 8.6, 5% variance) and 12 (peak 43.5 cM, Lod 10.4, 6% variance). For imipramine response in the TST as measured by residuals, regressing out the confounding contributions of body weight and basal TST, a significant QTL was mapped on chr 5 (peak 45 cM, Lod 7.9, 5% variance). Hence, the chr 5 locus affected both basal TST and antidepressant response, suggesting the existence of a pleiotropic locus influencing both stress vulnerability and antidepressant response. The human syntenic regions of this 20 cM Mus chr 5 region, namely 9p21-23 and 1p31-35, have been mapped as possible loci for bipolar disorder. Hence, these QTL provide promising loci for testing in stress-diathesis models of human psychiatric illness and antidepressant responsiveness.

A single locus from the SLE-prone strain BXSB is sufficient to drive autoantibody production and glomerulonephritis on a non-autoimmune background. *J.M. Rankin¹, J. Boyle², A.M. Calcagno Pizarelli¹, S. Izui³, B.J. Morley¹.* 1) Rheumatology Section, Eric Bywaters Centre, Faculty of Medicine, Imperial College, Hammersmith Campus, Du Cane Road, London W12 0NN, United Kingdom; 2) Histopathology Department, Hammersmith Hospital, Du Cane Road, London W12 0NN, United Kingdom; 3) Department of Pathology, Centre Medical Universitaire, Geneva, Switzerland.

Spontaneous murine models of Systemic Lupus Erythematosus (SLE) develop a high serum level of the retroviral envelope protein gp70. This is expressed at a low level in the serum of all murine strains, however only SLE-prone strains produce anti-gp70 autoantibodies. These form immune complexes with gp70 (gp70IC) and contribute to nephritis. In the BXSB model, the over-expression of gp70 and the production of gp70IC have been previously mapped to a single locus on chromosome 13 (*Bxs6*). To narrow down the *Bxs6* interval, we have carried out rare recombinant mapping using BXSB backcross mice. Recombinant events within the *Bxs6* interval were correlated with gp70 and gp70IC levels, allowing areas to be excluded. This approach successfully localised the *Bxs6* gene/s to a 16Mb interval, from the 35Mb region defined by the linkage study. We have bred a congenic line to determine whether *Bxs6* is sufficient on a non-autoimmune background for gp70 over-expression, gp70IC production and nephritis. These mice contain BXSB chromosome 13 (20Mb-117Mb) on a B10 background, in the presence of the BXSB Y chromosome autoimmune accelerator gene, *Yaa*. Congenic mice have a high serum level of gp70 and gp70IC, and develop a mild nephritis. These effects are not dependant on the presence of *Yaa*. The phenotype of these mice therefore replicates the linkage data, although levels lie midway between B10 and BXSB, implying that there must be other contributing factors in the BXSB genome. Interestingly, congenic mice also develop anti-ssDNA antibodies to which no linkage was found, although unlike the expression of gp70, this is dependant on the presence of *Yaa*. This is therefore a relatively simple system for the study of a single locus that, alone, directs autoantigen driven autoantibody production in a complex disease, but also demonstrates epistasis.

Examination of ELN as a candidate gene in the Utah Intracranial Aneurysm Pedigree Resource. *N.J. Berthelemy-Okazaki^{1,3}, N.J. Camp¹, J. Farnham¹, A. Thomas¹, K. Zhang², L.A. Cannon-Albright¹.* 1) Medical Informatics, University of Utah, Salt Lake City, UT; 2) Department of Ophthalmology and Visual Science, University of Utah, Salt Lake City, UT; 3) Zoology Department, Weber State University, Ogden, UT.

A linkage study of Intracranial Aneurysm in Japanese subjects implicated several candidate regions/genes, with the strongest evidence in the region of the elastin gene (ELN) (Onda et al., 2001). Analysis of ELN in the Japanese dataset suggested association of an ELN haplotype with IA risk. A subsequent linkage analysis of the ELN region in 14 high risk IA pedigrees from Utah significantly confirmed the linkage between IA and the ELN region (Farnham et al., 2003). We have investigated the ELN gene as a potential candidate gene responsible for the observed IA clustering and linkage in the Utah IA pedigree resource. One IA case from each pedigree who appeared to carry a segregating ELN -region haplotype was chosen for mutation screening. Two promoter regions, 34 exons and 3 3'UTR of the ELN gene were screened for variants by DHPLC. The variants were then sequenced. Each variant identified in an IA case was also screened for in one additional affected haplotype carrier relative and in one unaffected relative to test for segregation of the ELN variant with IA in the pedigree. Evidence for variants, indicated by differences in peak heights by WAVE analysis between controls and subjects, occurred in the promoter regions PM1, PM2, exons 4 and 6, and 3 3'UTR regions. These regions were sequenced and the variants identified. A rare variant in the promoter region PM3, present in the Japanese population, was not found in the Utah families. Variants in exon 6 and in one 3'UTR region were unique to Utah. The remaining variants were identical to the ones found in the Japanese population. None of these variants were present in the controls. No variation was found in the remaining 32 exons. There was no evidence for segregation of the ELN variants found in IA cases with the hypothesized segregating chromosome 7 haplotype examined. We saw no evidence supporting ELN as the gene responsible for familial IA in the Utah resource.

DNA Polymorphisms in two Paraoxonase Genes (PON1 and PON2) are independently associated with increased risk of Ischemic Stroke in Pakistani patients. *M.A. Bangash¹, D.A. Tregouet³, M. Saeed², H. Zuberi², E. Khan², S. Khanum², J. Sajid², A.H. Khan², A. Hasan², F. Abubakar², A.A. Khan², F. Fecto², W. Ahmed², T.U. Rehman², F. Cambien³, P.M. Frossard².* 1) School of Philosophy, University of Leeds,UK; 2) Biological and Biomedical Sciences, The Aga Khan University, Karachi, Pakistan; 3) INSERM U525, University Paris VI,France.

Stroke is the most common cause of disability and the third leading cause of death worldwide. As a highly heterogeneous disorder, it has several subtypes and multiple risk factors. To identify genes involved in Ischemic Stroke (IS) susceptibility, we investigated the association of functional Single-Nucleotide Polymorphisms (SNPs) in the Paraoxonase Genes (PON1 & PON2) and IS. PON1 enzyme prevents atherosclerosis by inhibiting oxidation of LDL, a role confirmed in functional studies and animal models. We analyzed 210 IS patients and 393 age, gender and ethnicity matched controls recruited from two tertiary care hospitals in Karachi. We genotyped 7 PON SNPs using PCR and restriction digestion assays. These included five PON1 (-909 G/C, -162 A/G, -108 C/T, Q192R, L55M) and two PON2 (148 A/G, 311 C/S) SNPs. We also determined serum PON activity by measuring rates of hydrolysis of paraoxon and phenylacetate. Among the seven SNPs, three were strongly associated with IS. Frequencies of 162A (0.47 vs. 0.40, $p < 10^{-2}$), 108T (0.57 vs. 0.46, $p < 10^{-3}$) and 148A (0.49 vs. 0.42, $p < 10^{-2}$) alleles were significantly higher in patients vs. controls. After adjustment for confounders, the 108TT genotype remained independently associated with a 2-fold increase risk of IS (OR= 2.4; 95% CI, 1.5-3.7; $p < 10^{-4}$). Subjects with the 108TT ($p < 10^{-2}$) and the 909CC ($p < 10^{-3}$) genotypes also had significantly higher phenylacetate activity. Haplotype analysis did not yield more information. Here we have reported novel association of two PON1 promoter region variants (-162 A/G and -108 C/T) and one PON2 (148 A/G) variant with increased risk of IS in our Pakistani patients. Our findings suggest an etiological role of PON1 and PON2 in the complex model of IS and further functional studies are needed to establish the causative mechanism.

Linkage disequilibrium mapping of the Endothelial Nitric Oxide Synthase (*NOS3*) gene and identification of *NOS3* SNPs associated with measures of endothelial function. S. Kathiresan^{1,4}, M.G. Larson¹, C.Y. Guo¹, J.A. Vita², G.F. Mitchell³, M.J. Keyes¹, C. Newton-Cheh^{1,4}, S. Musone⁴, A. Lochner⁴, J.A. Drake⁴, R.S. Vasan^{1,2}, D. Levy¹, C.J. O'Donnell^{1,4}, J.N. Hirschhorn⁴, E.J. Benjamin^{1,2}. 1) NHLBI's Framingham Heart Study, Framingham, MA; 2) Boston University School of Medicine, Boston, MA; 3) Cardiovascular Engineering, Holliston, MA; 4) Broad Institute, Cambridge, MA.

Targeted deletion of the *NOS3* gene in mice leads to endothelial dysfunction and hypertension. We sought to comprehensively characterize common single nucleotide polymorphisms (SNPs) and haplotypes at the *NOS3* locus and to test the hypothesis that common genetic variants at the *NOS3* locus influence brachial artery endothelial function in humans. We defined the linkage disequilibrium (LD) structure for common SNPs (minor allele frequency >5%) at the *NOS3* locus (genomic span 39 kb) using 33 SNPs in 93 individuals from 12 pedigrees of European ancestry. 17 SNPs that capture most of the common variation at *NOS3* were genotyped in 1446 unrelated Framingham Heart Study participants (50% women, mean age 62 years) in whom we measured brachial artery flow-mediated dilation (FMD) after 5 minutes of forearm ischemia. Sex-specific age- and multivariable-adjusted regression models were used to examine associations of SNPs and inferred haplotypes with FMD. An intronic SNP, rs1800781, was associated with FMD, in men only (nominal p-values 0.008, and 0.02 in age-adjusted and multivariable-adjusted analyses, respectively). Two additional intronic SNPs (rs3918169 and rs3918174) in tight LD with SNP rs1800781 ($D=1.0$ for both) were also associated with FMD in men (nominal p-value 0.02 for each SNP in fully adjusted model). A common multimarker haplotype in the 3' end of *NOS3* (45% frequency) was associated with impaired FMD (nominal p-value 0.002 in sex-pooled, multivariable-adjusted analyses). We defined a parsimonious set of SNPs that captures common genetic variation at the *NOS3* locus. Our results from a large community-based sample are consistent with the hypothesis that common *NOS3* sequence variants influence interindividual variability in endothelial function in men.

Determinants of the SNP marker density required for delineating major haplotypes. C. Ouyang¹, L. Geller¹, J.-E. Gustavson¹, P.D. Rodley¹, V.R.T. Gagalang¹, G.E. Rivas¹, C.G. Lundberg¹, A. Franck¹, H.-H. Lu¹, B. Shannon², C. Deckman¹, Y. Ding¹, M. Beaulieu³, S.D. Flanagan⁴, G.P. Larson¹, T.G. Krontiris¹. 1) Division of Molecular Medicine, Beckman Research Institute of the City of Hope, Duarte, CA; 2) Westridge School, Pasadena, CA; 3) Sequenom, Inc., San Diego, CA; 4) Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA.

The extent of linkage disequilibrium (LD) has been widely discussed as the major factor determining marker density for genome-wide disease mapping studies using single nucleotide polymorphisms (SNPs). We studied the molecular haplotype structure encompassing *PLA2G6* on chromosome 22 and *CDKN1A* on chromosome 6 in a European American sample. Both regions showed limited number of major haplotypes that could be tagged by haplotype-specific SNPs (SNPs having one allele specific to a major haplotype). We then performed computer simulations using a number of randomly chosen SNPs and determined the required marker density to delineate the major haplotypes faithfully, as if the haplotype structure were unknown to us. Interestingly, the required marker density at the two loci differed significantly, from 1 SNP per 10 kb at *PLA2G6* to over 10 SNPs per 10 kb at *CDKN1A*. The latter value was directly related to an uneven distribution across major haplotypes of haplotype-specific SNPs. Our findings suggest that, besides the extent of LD, additional factors that include the distribution of haplotype-specific SNPs and the number of major haplotypes together determine the useful SNP density for a genome-wide haplotype map. A significant portion of the human genome may require a much higher density than that recommended for the initial HapMap build.

Genome-wide linkage analysis of allergic rhinoconjunctivitis in a Swedish population. *M. Bradley*^{1,2}, *B. Liming*², *C. Söderhäll*², *C-F. Wahlgren*¹, *I. Kockum*², *M. Nordenskjöld*². 1) Dept Dermatology, Karolinska Inst, Stockholm, Sweden; 2) Dept Molecular Medicin, Karolinska Inst, Stockholm, Sweden.

Allergic rhinoconjunctivitis is a common complex disorder characterized by itching and irritation in the nose, bouts of sneezing, watery rhinorrhoea, nasal congestion and itchy eyes with tears and swelling. Like other atopic disorders such as allergic asthma and atopic dermatitis, the development involves complex interactions of genes and environmental factors. To identify susceptibility loci for allergic rhinoconjunctivitis, we conducted a genome-wide linkage analysis using a non-parametric, affected-relative-pair method. The 250 families used were collected originally for an atopic dermatitis linkage study. Two regions showed favour in evidence of linkage to allergic rhinoconjunctivitis: 3q13 (D3S1278: LOD=1.67, p0.003), 4q34-35 (D4S1652: LOD=1.49, 0.005). In addition, five regions showed weaker evidence in favour of linkage: 6p22-24 (D6S1959: LOD=1.39, p0.006), 9p12-q11 (D9S1118: LOD=1.15, p0.02), 9q33.2-34.3 (D9S915: LOD=1.29, p0.01), 17q11.2 (D17S1294: LOD=1.13, p0.02) and 18q12 (D18S535: LOD=1.01, p0.02). In single-point analysis, one locus on chromosome 3 close to marker D3S1278 reaches the suggestive level (LOD=2.28, $p6 \times 10^{-4}$ while one on chromosome 17 close to marker D17S921 almost reaches this level (LOD=2.17, 8×10^{-4}). Our results support the linkage to allergic rhinoconjunctivitis on 3q12, 6p24-p23 and 9q34.3 shown in previous investigations.

Fine mapping of the critical multiple sclerosis region on 5p12-p14. *D.O. Bronnikov^{1,2}, S. Kallio², J.S. Saarela², H.M.F. Riise Stensland³, P. Pajukanta¹, A. Palotie^{1,2}, L. Peltonen^{1,2}.* 1) Department of Human Genetics, David Geffen School of Medicine at UCLA, University of California, Los Angeles, California, USA; 2) National Institute of Public Health, Department of Molecular Medicine, Helsinki, Finland; 3) Medical Biochemistry, University of Troms, Troms, Norway.

Multiple Sclerosis (MS) is a chronic inflammatory disorder of Central Nervous System; it is a multifactorial trait with a complex mode of inheritance. Highly prevalent in Caucasians of Northern European origin (50-100/10⁵), MS is a common cause of acquired neurological disability in 20-40 year olds. Apart from the finding of strong MS linkage on Major Histocompatibility Complex, only three linked loci, located on chromosomes 5, 17 and 19, overlapped between previous genomewide scans, all replicated in Finnish study sample. The linked region on chromosome 5p is syntenic to an EAE locus on mouse chromosome 15. We restricted the initial 40cM region in a follow-up study to 9cM on 5p12-p14 using extended Finnish MS families. A set of 65 SNPs, located in coding regions and regulatory elements of known regional genes and putative transcripts, confirmed by RT-PCR from total RNA, and also regions of high synteny to mouse, were genotyped in 22 multiplex Finnish pedigrees and 42 MS trios originating from high MS-risk sub-isolate on Western coastline of Finland. Two regions, located within CTNND2 and LOC90268 genes, had a number of markers showing suggestive evidence for association. Pairwise LD between the markers located within 500kb to each other was monitored using GenePOP and several haplotype blocks, the longest one stretching over 200kb, were observed. The most informative markers belonging to these genomic regions were genotyped in additional 439 Finnish MS trios in order to replicate our initial findings. Stratification based on the origin of the studied families resulted in p-value 0.000993 with the recessive model of LD given linkage test using the Pseudomarker program. To gain some functional evidence for the associated genes, we monitored for expression differences of the genes located in the MS critical region in peripheral blood circulating T-cell fractions collected from MS patients and their healthy relatives.

A SNP in an interferon-stimulated response element in the promoter of the CHRNA1 gene, encoding the alpha-subunit of the muscle acetylcholine receptor, is associated with autoimmune myasthenia gravis. *M. Giraud¹, B. Eymard², C. Tranchant³, D. Beeson⁴, N. Willcox⁴, P. Gajdos⁵, HJ. Garchon¹.* 1) INSERM U580, Hôpital Necker, Paris, France; 2) Hôpital de la Salpêtrière, Paris, France; 3) Hôpital Civil, Strasbourg, France; 4) The Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, United Kingdom; 5) Hôpital Raymond Poincaré, Garches, France.

Myasthenia gravis (MG) is an autoimmune disease affecting neuromuscular transmission, where the prime self-antigen, the muscle acetylcholine receptor (AChR), has been unambiguously identified. The AChR alpha-chain (encoded by the CHRNA1 gene) is the target of most pathogenic autoantibodies. We had previously shown an association of CHRNA1 with MG susceptibility. Here, we have exhaustively characterized its polymorphism by re-sequencing 19.9 kb of genomic sequence encompassing the locus in 16 individuals. We have uncovered 37 new polymorphisms and confirmed another 33. Apart from one, a synonymous substitution in exon 7, all are located in non-exonic sequences. The genotyping of 35 additional single nucleotide polymorphisms (SNPs), located up to 50 kb on each side of CHRNA1, allowed us to identify an island of linkage disequilibrium (LD) spanning 40 kb, including CHRNA1 only and excluding neighbor genes. The pattern of allelic associations of common polymorphisms was characterized and their effects on the phenotype of MG patients were investigated. We disclosed a highly significant association of a group of 12 in-phase polymorphisms with an early onset of the disease (<20 years) in 186 French MG patients compared to 154 population-matched controls (OR = 3.5; P<0.00006). Remarkably, one SNP within this group, located in the promoter region, modifies a typical IFN-stimulated response element (ISRE) and potentially affects the transcriptional efficiency of CHRNA1 in immune cells. Association of this SNP was replicated in the British population, in a sample of 151 unrelated patients and 109 controls (P<0.025). This SNP is therefore a good candidate to explain the association of its group with MG and to undertake functional studies.

Linkage and Candidate Gene Analysis for Developmental Dyslexia on Chromosome 18. *T.S. Scerri¹, I.L. MacPhie¹, S. Paracchini¹, J. Walter², A.J. Richardson², C. Francks¹, J.F. Stein², A.P. Monaco¹.* 1) Wellcome Trust Centre for Human Genetics, University of Oxford, United Kingdom; 2) Department of Physiology, University of Oxford, United Kingdom.

Developmental dyslexia (DD) is a specific and significant impairment in reading ability despite adequate educational opportunity and intelligence, and is diagnosed in approximately 5% of school-aged children. There is strong evidence from twin and family-based studies that both environmental and genetic components play a role in the aetiology of DD, thus making it a multifactorial disorder. Several genome-wide linkage studies have identified genomic regions that may harbour quantitative trait loci (QTLs) for DD on chromosomes 2, 6, 11, 15 and 18. We first reported linkage of a DD QTL at 18p11.2 in three large independent family samples, including two from the United Kingdom (UK) and one from the United States. We have now collected a third independent UK sample of 91 families. Our combined UK sample now consists of 264 nuclear families, each with at least one dyslexic proband, that have all been genotyped for 54 polymorphic microsatellite markers across the entire length of chromosome 18, with an average inter-marker spacing of ~2.3 cM. The evidence for linkage of DD to chromosome 18 has been reassessed with this denser set of markers in our combined sample of UK families. Using four different methods of linkage analysis (Haseman-Elston, variance components, DeFries-Fulker and multivariate), and an up-to-date deCODE genetic map, we confirm the existence of the 18p11.2 QTL, and present evidence for a possible second QTL at 18q12.2. Mutation screening of the exons and predicted promoters of candidate genes within the vicinity of the 18p11.2 and 18q12.2 QTLs is currently being conducted by temperature modulated heteroduplex analysis. Thus far, we have not identified a mutation with an obvious disruptive affect within any of the candidate genes. Nevertheless, a number of single nucleotide polymorphisms (SNPs) have been identified. A selection of these, along with other SNPs reported in the online databases, are being genotyped and will be used to test for association to DD in our set of UK families.

RGS4 is associated with systemic lupus erythematosus (SLE) at 1q23.3. *H. Wu*^{1,2}, *R.M. Cantor*³, *J.M. Grossman*¹, *E. Park*¹, *A.A. Rumbin*¹, *D.J. Wallace*⁴, *B.H. Hahn*¹, *B.P. Tsao*¹. 1) Dept. of Medicine, Div. of Rheumatology, UCLA, Los Angeles, CA; 2) Dept. of Rheumatology, Ren Ji hospital, Shanghai Second Medical University, Shanghai, China 200001; 3) Dept. of Human Genetics and Pediatrics, UCLA, Los Angeles, CA; 4) Cedar-Sinai Research Institute, Los Angeles, CA.

SLE is a genetically complex, multifactorial autoimmune disease, and cumulative studies implicate multiple factors in its pathogenesis. In a Caucasian SLE cohort with 67 affected sibpairs and 224 simplex families, all patients fulfilled the American College of Rheumatology criteria for SLE. We previously found linkage of SLE to 1q23.3 in Caucasians (peak at D1S1677, 175 cM, NPL = 2.44) and association of SLE in the linked region with alleles of D1S2844 (160.1 Mb, allele-wise $p = 0.0009$). To further localize the putative SLE susceptibility gene(s), we genotyped 26 informative SNPs (frequency >10%) spanning a 1Mb region flanking D1S2844 (159.8-160.8 Mb) using the Sequenom, Pyrosequencing and TaqMan assays. All genotype distributions were consistent with Hardy-Weinberg equilibrium. We tested the 26 SNPs for LD with the SLE associated multiallelic marker D1S2844, and found that one SNP within the regulator of G-protein signaling 4 gene (RGS4) (rs2940251, $p = 0.0071$) could explain the original association with SLE. Preferential transmission of SNPs to SLE patients for 6 individual SNPs within RGS4 was tested using the Transmission Disequilibrium Test (TDT), and the TRANSMIT program which uses an EM algorithm was used to test for preferential transmission of SNP haplotypes. Preferential transmission of the major alleles of 2 individual RGS4 SNPs (rs10759, rs2940251) was observed (T: UT = 78: 50, $p = 0.013$ and 85: 61, $p = 0.047$ respectively). These 2 SNPs are in strong LD ($D = 0.85$, $p < 0.00001$) with each other. Haplotypes spanning 13 kb formed by 5 RGS4 SNPs (rs951436, rs951439, rs2661319, rs2842030 and rs10759) were associated with SLE (global $p = 0.049$, $p = 0.0001$ for the haplotype formed by all the major alleles). RGS4, expressed in multiple tissues and cell types, may modulate signal transduction by increasing the GTPase activity of G protein alpha subunits to contribute to the development of SLE.

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A third locus for isolated autosomal dominant optic atrophy. *F. Barbet, S. Gerber, A. Munnich, JM. Rozet, J. Kaplan.* Genetics, INSERM U393, Paris, Cedex 15, France.

The aim of this study was to localise the gene responsible for autosomal dominant optic atrophy in two large multiplex families in which OPA1, on chromosome 3q28, and OPA4, on chromosome 18q12, were excluded. A 10 cM genome-wide search for linkage was performed in both families using fluorescent microsatellites. In each family, an haploidentity was found for all affected patients in the same chromosomal region. The maximal lod-score at this locus was equal to 3.33 with a penetrance estimated to 0.80. The minimal genetic interval containing the disease gene was equal to 3 cM when both families were considered. It has been reported that OPA1 mutations are responsible for the majority of autosomal dominant optic atrophies characterized by a large variability of severity and an incomplete penetrance (0.70). In addition, the gene responsible for the disease in one family has been reported on chromosome 18q12 but this gene may account for only a few pedigrees. This work demonstrates that a third locus exists which may account for some families affected with an isolated autosomal dominant optic atrophy, clinically different from the OPA1 phenotype. Several candidate genes involved in mitochondrial function have been identified in this novel genetic interval and are currently under investigation.

Genetic evaluation of a large family with optic atrophy. *H.K. Peters*¹, *M.V. Zaragoza*¹, *P.L. Flodman*¹, *A.B. Fulton*³, *G. Cox*⁴, *T. Huang*^{1,2}. 1) Dept. of Pediatrics, Division of Human Genetics, Univ California, Irvine, Orange, CA; 2) Dept. of Developmental and Cell Biology, Univ California, Irvine, Irvine, CA; 3) Dept. of Ophthalmology, The Children's Hospital, Boston, MA; 4) Dept. of Genetics, The Children's Hospital, Boston, MA.

Optic Atrophy (OPA) is the progressive degeneration of the retinal ganglion cells resulting in central vision loss, central visual field deficits and color blindness. The condition may be isolated or a feature of a genetic syndrome. Familial OPA shows several inheritance patterns including autosomal dominant, autosomal recessive and X-linked. The most common type of inherited OPA is autosomal dominant optic atrophy (ADOA) with an estimated incidence of 1:12,000 to 1:50,000. Typically, ADOA presents in the first decade of life and exhibits both intrafamilial and interfamilial variability. Two loci, the major locus *OPA1* on 3q28-29 and a second locus *OPA4* on 18q12.2-q12.3, have been shown to contribute to ADOA. To date, a vast number of mutations have been described in patients with ADOA in the *OPA1* gene that encodes a dynamin-related GTP protein that localizes to the mitochondria. The X-linked OPA locus, *OPA2*, has been linked to Xp11.4-p11.2 in a large Dutch pedigree. In addition to OPA, affected males displayed other neurological findings including mental retardation. Female carriers were phenotypically normal.

This report describes the clinical evaluation and genetic studies of a large, five-generation family with isolated optic atrophy in multiple affected members. Interestingly, affected males appear to present at an earlier age compared to affected females. This finding is similar to three families previously described with sex-influenced severity. To identify genetic factors in OPA, we examine the family for genetic linkage to 3q28-q29 and Xp11.4-p11.2. Elucidation of the genetic factors in this family will contribute to the understanding of hereditary optic neuropathies and modifying factors of sex-influenced traits.

A Genomewide Scan for Quantitative Trait Loci Linked to Waist Circumference in Mexican Americans. *R. Arya*¹, *J. Schneider*², *S. Puppala*², *T. Dyer*², *S. Fowler*¹, *L. Almasy*², *J. Blangero*², *M.P. Stern*¹, *R. Duggirala*². 1) Department of Medicine/Clinical Epidemiology, University of Texas Health Science Center, San Antonio, TX; 2) Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX.

Epidemiological studies show a rapid increase in obesity, especially abdominal obesity worldwide. Waist circumference (WC) is an important surrogate measure of abdominal fat deposition and more strongly associated with risk factors of cardiovascular disease than body mass index (BMI). Although, several quantitative trait loci (QTLs) influencing obesity-related traits have been reported, specific QTLs that independently and/or pleiotropically influence WC have not been identified in Mexican Americans, a population characterized by increased adiposity and a more centralized distribution of body fat. In this study, we performed a genome scan of WC, adjusted for BMI using a variance component linkage approach to identify loci influencing WC. We analyzed phenotypic data from 715 participants in the San Antonio Family Diabetes/Gallbladder Study (SAFDGS) (27% diabetics, 60% females, mean age [SD] 45.16 years, mean WC [SD] = 997.4/166.8 mm, mean BMI (kg/m²) [SD] = 30.9/6.9) distributed across 39 low-income Mexican American pedigrees, for whom a 10 cM map is available. We conducted a multipoint variance component linkage analysis using SOLAR. WC values were log transformed to minimize the problem of non-normality. After accounting for the covariate effects of age, sex, type 2 diabetes, and BMI, WC was heritable (N=713, h² SE = 0.34/0.08; p < 0.0001). In linkage analysis, five genetic regions on chromosomes 1, 9, 10, 11, and 17 were found to show some evidence for linkage (LOD 1.5) to WC, with the highest LOD of 2.8 (p = 0.0004) occurring between markers D1S547-D1S1609 on chromosome 1q43-44. This marker region is located within 35 cM telomeric to regions previously reported to be linked to glucose and BMI-related phenotypes. These findings suggest that both unique and common susceptibility loci may be influencing WC in Mexican Americans. This study is supported in part by NIH grants DK53889, DK42273, and DK47482.

Experimental pain sensitivity is not influenced by common haplotypes of DREAM, transcriptional repressor of the prodynorphin gene. *B. Buzas*^{1,2}, *I. Lorincz*^{1,2}, *I. Belfer*^{1,2}, *L. Diatchenko*³, *W. Maixner*³, *D. Goldman*², *M.B. Max*¹.
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DREAM (downstream regulatory element antagonistic modulator) is a repressor of prodynorphin transcription. Calcium inhibits DREAM binding to DRE leading to increased transcription. DREAM knockout mice display reduced pain responses to acute painful stimuli and show diminished pain behavior in chronic neuropathic and inflammatory conditions. This reduced nociception is attributed to the removal of the constitutive repression by DREAM of prodynorphin expression in the spinal cord. Thus genetic polymorphisms of DREAM may influence prodynorphin expression and nociceptive sensitivity. Informative haplotypes can capture the effects on phenotype of moderately abundant, effective alleles, even if these are unknown. By genotyping 24 SNPs, we determined the haplotype block structure of the genomic region of DREAM (90 kb, 2q21.1) in five populations (U.S. Caucasian, Finn, African American, Plains and Southwest Native Americans). In all populations there was very high pairwise LD between SNP markers 1-20 which placed 80% of the gene in one strong LD block, and evidence for extended superblock structure was also found. As expected common haplotypes and their frequencies showed similarity between the U.S. Caucasian and Finnish populations, and also between the two Native American populations, however less similarity was evident between the Native American and two Caucasian populations. We identified 4 haplotype tag SNPs that were sufficient to capture haplotype diversity in the Caucasians. These SNPs were genotyped in 231 Caucasians, and the haplotypes were analyzed for association with pain perception. 16 different measures of thermal, pressure, and ischemic pain measures were taken in each individual, and were standardized to unit normal deviates (z-scores). No statistically significant correlation between individual haplotypes and diplotypes and pain perception has been found indicating that genetic polymorphisms in DREAM do not significantly contribute to interindividual differences in pain perception.

Identification of Susceptibility Genes in Systemic Lupus Erythematosus. *A. Hellquist¹, C.M. Lindgren¹, P. Onkamo⁴, L. Berglind¹, S. Koskenmies², M. Zucchelli¹, E. Widen³, H. Julkunen⁵, J. Kere^{1, 2, 3}.* 1) Department of Bioscience, Karolinska Institutet, Stockholm, Sweden; 2) Department of Medical Genetics, University of Helsinki, Finland; 3) Finnish Genome Center, Helsinki, Finland; 4) Helsinki Institute for Information Technology, Basic Research Unit Department of Computer Science, University of Helsinki, Finland; 5) Department of Internal Medicine, Peijas Hospital, Helsinki University Hospital, Finland.

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune inflammatory disease with unclear etiology, however strong heritability has been shown. Several candidate susceptibility loci have been identified, but most of the genetic background remains unexplained as yet. In a previous genome-wide scan with 35 Finnish families affected with SLE, we detected suggestive linkage on chromosomes 5p, 6q25-q27, 14q21-q23 as well as HLA on 6p (Koskenmies et al 2004a). Further, an excess sharing of a haplotype on 14q and excess transmission of a haplotype on 6q were shown after additional markers were added at 1 cM density across the suggestive regions of linkage on chromosomes 6q23- q27 and 14q21-q23 (Koskenmies et al 2004b). We have now used a dense microsatellite and single nucleotide polymorphism (SNP) mapping approach to further limit the areas of interest on chromosomes 6q25- q27 and 14q21-q23 (altogether, 31 new microsatellites and 26 new SNPs at 44 400 kb density) by linkage disequilibrium (LD) mapping. Our association mapping results using the HPM algorithm (Toivonen et al 2000) suggest that both chromosomal regions are of interest for gene identification, and associated segments of 1.5 Mb have been identified with present resolution of markers. The project continues in an attempt to identify the respective susceptibility genes. Koskenmies S et al. *J Med Genet.* 2004a:Jan; 41(1):e2-5 Koskenmies S et al. *Eur J Hum Genet.* 2004b:Mar 31 Toivonen H T et al. *Am J Hum Genet* 2000:67(1):133-45.

Large scale Family Based Association Study in Left Ventricular Outflow Tract Malformations: A Candidate Developmental Pathway Approach. *K. McBride¹, S. Fernbach¹, L. Molinari¹, A. Menesses¹, T. Ho¹, R. Pignatelli¹, N. Kaplan³, R.A. Gibbs¹, T. Willis², M. Faham², P. Hardenbol², M. Moorhead², J.A. Towbin¹, J.W. Belmont¹.* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) ParAllele Bioscience, South San Francisco, CA; 3) NIEHS.

Congenital left ventricular outflow tract (LVOT) malformations are common complex heart malformations that are a leading cause of infant mortality. Evidence from multiple sources suggests a strong genetic contribution to the etiology of LVOTO defects ($\lambda_S \approx 32$), but the gene(s) involved have not been elucidated. The pattern of inheritance is most likely oligogenic. We designed a large scale candidate gene association study using family-based controls (case-parent trios). We selected 26 groups of genes based on data from animal and in vitro models that indicate the requirement for those pathways in cardiac development. Single nucleotide polymorphisms within genes in these pathways were selected from dbSNP with an average spacing of 1.5 kb. A total of 1977 SNPs in 200 genes produced working assays using a highly multiplexed, microarray based system (Molecular Inversion Probe, ParAllele Biosciences). We genotyped 215 affected case-parent trios producing a total of 1.3 million genotypes. Transmission disequilibrium tests and Hardy Weinberg equilibrium tests were performed on all SNPs. Dependence between markers was assessed by the linkage disequilibrium metric D in HAPLOVIEW and association of haplotypes was assessed using TRANSMIT. Control for multiple testing was performed utilizing a False Detection Rate, or q value, of 0.20, for each pathway group of SNPs. In all 15 genes in 8 pathways showed FDR $q < 0.2$. We observed significant association of markers in four genes - ERBB4, ERBB3, ERBB1, and Neuregulin - within the same ligand-receptor pathway. These results demonstrate the strength of the pathway approach, and support the probable role of ERBB class receptors in LVOTO defects.

Genome Wide Linkage Analysis of a Novel Hereditary Progressive Hyperpigmentation Provides Evidence for Linkage to Chromosome 11. *T. Vogt*¹, *M. Amyere*², *M. Vikkula*². 1) Department of Dermatology, University of Regensburg, Regensburg, Germany; 2) Laboratory of Human Molecular Genetics, Christian de Duve Institute of Cellular Pathology & Université catholique de Louvain, Brussels, Belgium.

Familial progressive hypermelanosis is a new variant of a hereditary pigmentation disorders without associated symptoms. This phenotype has so far been observed only in three families, all living in close proximity in a small town in south-east of Germany. The phenotype, with an autosomal dominant inheritance, consists of progressive diffuse, partly blotchy hyperpigmentation, multiple caf-au-lait spots, intermingled with scattered hypopigmented appearing maculae, and lentigenes. The restricted area of occurrence of the disease and the extreme rarity of the phenotype suggest a common origin and thus a founder effect for this genetic defect. Based on this hypothesis, we performed a genome-wide linkage analysis in the three families using SNP genotyping assay, the GeneChip Human Mapping 10K Array. The results demonstrate the presence of significant linkage peak with maximal LOD of 2.156 assuming the smallest genetic distance between the three families, on the long arm of chromosome 11. All affected individuals shared the same haplotype in this area. This finding suggests that a gene involved in melanin distribution is located on chromosome 11. (<http://www.icp.ucl.ac.be/vikkula>) (vikkula@bchm.ucl.ac.be).

Identification of two novel mitochondrial DNA changes (T1243C and T1291C) in the 12S rRNA associated with nonsyndromic hearing loss. *E. Ballana*¹, *E. Morales*², *P. Gasparini*³, *E. Gean*⁴, *B. Montserrat*¹, *X. Estivill*¹. 1) Center for Genomic Regulation, Barcelona; 2) Centro Nacional de Genética Médica, La Habana; 3) Tigem, Naples; 4) Hospital St Joan de Deu, Barcelona.

Mutations in the mtDNA have been identified as a cause of syndromic and nonsyndromic forms of hearing loss. Among the nonsyndromic deafness-causing mtDNA mutations are the delT961Cn and the A1555G in the 12S rRNA gene and four mutations in the tRNA^{Ser}(UCN) gene (A7445G, 7472insC, T7510C, T7511C). The A1555G mutation in the 12S rRNA gene has been associated with aminoglycoside-induced and nonsyndromic deafness in many families. The resulting phenotype varies among family members, ranging from severe deafness, to moderate progressive hearing loss and normal hearing and it is thought to be due to the interaction between other genetic and environmental factors. A total of 455 families or deaf individuals have been collected to test them for the presence of mutations in known deafness-causing genes. In those cases where the familial history was consistent with maternal inheritance, the samples were analyzed for mutations in the 12S rRNA gene. Mutation A1555G was found in 68 cases, confirming the high frequency of this mutation in Spain. A novel mutation T1291C was identified in a three-generation Cuban family with sensorineural progressive hearing loss and without previous history of aminoglycoside exposure. Audiometric evaluation showed a more severe hearing loss at high frequencies and a wide range in the age at onset of varying from 7 to 40 years. Another non-reported mutation, T1243C, was detected in one Spanish and one Italian sample, affected of sensorineural deafness, without aminoglycoside exposure, but with differences in ages at onset. Thus, the screening of the 12S rRNA gene for various mutations, should be considered in those patients with a family history consistent with maternal inheritance. As hypothesized for the A1555G mutation, the wide variability of the underlying phenotype also found in the new identified mutations, suggest the implication of other environmental or genetic factors, which could contribute to the severity and age of onset of hearing loss.

Human Trmu related to mitochondrial tRNA modification is a modifier factor for the phenotypic expression of the deafness-associated mitochondrial 12S rRNA mutation. *M. Guan*^{1,7}, *Q. Yan*¹, *X. Li*¹, *Y. Bykhovskaya*², *E. Mengesha*², *N. Umeda*³, *P. Hajek*⁴, *R. Li*¹, *J. Peters*¹, *M. Shohat*⁵, *X. Estivill*⁶, *T. Suzuki*³, *K. Watanabe*³, *N. Fischel-Ghodsian*². 1) Div Human Genetics, Children's Hosp Medical Ctr, Cincinnati, OH; 2) 2Medical Genetics Institute, Ahmanson Department of Pediatrics, Steven Spielberg Pediatric Research Center, Cedars-Sinai Medical Center and David Geffen School of Medicine at UCLA, Los Angeles, California, 90049, USA; 3) Department of Integrated Biosciences, Graduate School of Frontier Sciences, University of Tokyo, Kashiwa, Chiba, 277-8562, Japan; 4) Division of Biology, California Institute of Technology, Pasadena, California 91125, USA; 5) Department of Pediatrics and Medical Genetics, Basil and Gerald Felsenstein Medical Research Center, Tel Aviv University Medical School, Petah Tikva, Israel; 6) Genes and Disease Program, Center for Genomics Regulation, Barcelona Biomedical Research Park, Barcelona, Spain; 7) Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45229-3039, USA.

Nuclear modifier genes have been proposed to modulate the phenotypic expression of deafness-associated 12S rRNA A1555G mutation. We report here the identification of TRMU encoding a highly conserved mitochondrial protein related to tRNA modification. TRMU is ubiquitously expressed and more abundantly in tissues of high metabolic rates. We show that in families with A1555G mutation there is highly suggestive linkage and linkage disequilibrium between microsatellite markers adjacent to TRMU and the presence of hearing loss. Sequencing of TRMU in deaf members of these families revealed a missense mutation (G28T) altering an invariant amino-acid residue (A10S) in the mitochondrial targeting sequence of the gene. Strikingly, the homozygous mutation causes marked defect in mitochondrial tRNA metabolism but does not affect importing and processing of Trmu precursors. The resultant biochemical defects aggravate the mitochondrial dysfunction associated with A1555G mutation. These findings indicate that truncated TRMU, acting as a mitochondrial tRNA modifier factor, modulates the phenotypic manifestation of deafness-associated A1555G mutation.

Molecular and clinical characterization of a Japanese family with maternally inherited nonsyndromic hearing loss. *R. Li¹, K. Ishikawa², L. Yang¹, Y. Tamagawa², K. Ichimura², M.X. Guan^{1,3}.* 1) Dept Human Genetics, Cincinnati Children's Hosp, Cincinnati, OH; 2) Department of Otolaryngology, Jichi Medical School, Minamikawachi, Tochigi, Japan; 3) Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45229, USA.

The T7511C mutation in the mitochondrial tRNA^{Ser}(UCN) gene has been identified to be associated with nonsyndromic deafness in several families from different ethnic groups including Japanese and African-American families. We report here the characterization of a Japanese family with maternally transmitted non-syndromic deafness. 14 of 21 matrilineal relatives in this family exhibited early or late-onset/progressive but non-congenital hearing impairment with a wide range of severity, ranging from severe to normal hearing. The age of onset varies from 3 years to 30 years. Sequence analysis of the complete mitochondrial genome in this pedigree revealed the presence of T7511C mutation and other variants. However, none of these mtDNA variants are evolutionarily conserved and implicated to have significantly functional consequence. Incomplete penetrance in this family indicates the involvement of modulatory factors including other mtDNA mutations/polymorphisms and/or nuclear backgrounds in the phenotypic expression of hearing impairment associated with the T7511C mutation. In particular, the absence of the ND1 T3308C and tRNA^{Ala} T5655C mutations in the Japanese family with the T7511C mutation but the presence of these mtDNA mutations in the African-American family seems to account for different penetrance between two pedigrees. However, our molecular and genetic data showed that variants of GJB2 gene may not act as a modifier factor for the phenotypic expression of the T7511C mutation in this Japanese family.

Molecular analysis of the mitochondrial 12S rRNA in Chinese subjects with hearing loss. *W.Y. Young¹, Q. Wang¹, L. Zhao¹, R. Li², M.X. Guan^{1,2}.* 1) Institute of Otolaryngology, Chinese PLA General Hospital, Beijing, China; 2) Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OHIO.

Mutations in mitochondrial DNA (mtDNA) have been found to be one of the most important causes of sensorineural hearing loss. We report here a systematically mutational screening of the mitochondrial 12S rRNA and gene in 276 sporadic Chinese subjects with hearing impairments recruited from the Otology Clinic of Chinese PLA General Hospital. We showed that the frequency of the A1555G mutation is 1.8 % in this Chinese population with hearing impairment. The deafness-associated homoplasmic C1095T mutation in the 12S rRNA gene has been identified in two subjects with aminoglycoside induced deafness and one subject with auditory neuropathy. One subject carries the 961insC mutation. Furthermore, two variants A827G and T1005C in the 12S rRNA gene, which are localized at highly conserved sites, have been found in the affected individuals but absent in 118 controls, indicating that they may play a role in the pathogenesis of deafness. In addition, ten other variants have been found in this Chinese deaf population. These data strongly suggest that the mitochondrial 12S rRNA gene is the hot spot for deafness-associated mutations in the Chinese population.

Biochemical characterization of the mitochondrial 12S rRNA C1494T mutation associated with maternally inherited aminoglycoside-induced and non-syndromic deafness in a large Chinese family. *H. Zhao*^{1,2}, *R. Li*¹, *Q. Yan*¹, *X. Li*¹, *Q. Wang*², *D. Han*², *W.Y. Young*², *M.X. Guan*¹. 1) Human Genetics, Cincinnati Children's Hospital, Cincinnati, OH; 2) Department of Otolaryngology, Chinese PLA General Hospital, Beijing, China.

Biochemical characterization of mitochondrial 12S rRNA C1494T mutation associated with maternally inherited aminoglycoside-induced and non-syndromic deafness has been carried out by using cybrids constructed by transferring mitochondria from lymphoblastoid cell lines derived from the Chinese family into human mtDNA-less (o) cells. 15 cybrids derived from five matrilineal relative of the Chinese family carrying the C1494T mutation, exhibited a significant decrease in the rate of mitochondrial protein synthesis, respiration and growth, compared with 9 control cybrids derived from three controls lacking the mutation. Furthermore, exposure to high concentration of paromomycin or neomycin caused a uniformed and significant average increase in doubling time in 15 cybrid cell lines derived from three symptomatic and two asymptomatic individuals in this family carrying the C1494T mutation, when compared to nine cybrids derived from three control cell lines. These results provide a direct biochemical evidence that the C1494T mutation is the novel mitochondrial DNA mutation associated with aminoglycoside-induced and nonsyndromic deafness. This data also suggest that the nuclear background plays a determining role in the aminoglycoside toxicity.

A NRG1 signaling pathway through Fyn kinase modulates NMDA-R tyrosine phosphorylation: Genetic interaction and relevance to the pathophysiology of schizophrenia. *T. Andresson, S. Haverfield-Gross, M. Bjarnadottir, H. Stefansson, S. Bruun, V. Helgason, A. Sigmundsson, D. Firth, B. Nielsen, A. Kong, J. Gulcher, K. Stefansson, M. Gurney.* deCODE genetics, Reykjavik, Iceland.

Schizophrenia is a complex disease of unknown etiology affecting 0.5-1% of the population. The disease typically is treated with dopamine receptor type-2 antagonists such as haloperidol or with so-called atypical antipsychotic drugs such as clozapine. We previously identified Neuregulin1 (NRG1) as a gene contributing to risk of schizophrenia. Furthermore, we showed that NRG-/+ mutant mice display behavioral abnormalities that are reversed by clozapine. The hypothesis that schizophrenia may result from NMDA-R hypofunction has gained increasing support in recent years. p59Fyn, a src-family kinase, has previously been shown to phosphorylate the NMDA-R subunit NR2B on tyrosine 1472 (Y1472), thereby increasing NMDA-R channel open time and channel open probability. We have identified p59Fyn as an ErbB4 binding protein (ErbB4 is the predominant receptor for NRG1 on CNS neurons) and have demonstrated that p59Fyn kinase is activated in response to NRG1 signaling through ErbB4. Stimulation of ErbB4 by NRG1 in neuroblastoma cells leads to increased phosphorylation of NR2B Y1472. Furthermore, we find that NR2B Y1472 is hypo-phosphorylated in NRG-/+ and ErbB4-/+ mutant mice. The defect in NR2B Y1472 phosphorylation in NRG-/+ mutant mice can be reversed by clozapine at a dose that reverses the behavioral abnormality. Thus, a biochemical defect in NMDA-R subunit tyrosine phosphorylation is linked genetically and biochemically to the NRG1 signaling pathway in schizophrenia and this defect can be reversed by an atypical antipsychotic drug. In Iceland, individuals have a greater risk of developing schizophrenia if longer alleles of the microsatellite marker D16S407 in the GRIN2A promoter and the NRG1 SNP at-risk haplotype coincide. Therefore our combined genetic and biochemical data are in keeping with the glutamate hypothesis of schizophrenia. Subtle misregulation of NRG1 signaling may alter NMDA receptor modulation increasing susceptibility to schizophrenia.

A Role For Estrogen Signaling in Schizophrenia. *S.E. Dobrin, K. Ramsey, J. Parod, D. Hu-Lince, W. Liang, D.A. Stephan.* Neurogenomics Division, TGen, The Translational Genomics Research Institute Phoenix, AZ.

Schizophrenia is a frequent, disabling disease of variable expression and unknown etiology. Symptoms include hallucinations, delusions, paranoia, lack of motivation and anhedonia. The prevalence of schizophrenia is estimated to be about 1% worldwide. Ordinarily, the onset of this illness occurs in adolescence or early adulthood but has been observed in later stages of life as well. In all likelihood, schizophrenia is etiologically heterogeneous. Classical segregation analysis has been unable to identify a single mode of inheritance to explain the familiarity of schizophrenia. However, analyses suggest a complex and multigenic susceptibility to schizophrenia involving both genes and experiences in a majority of the cases. To date, no gene of major significance has been identified for schizophrenia using traditional genetic approaches. Here we present data from expression profiling RNA extracted from the prefrontal cortex of 70 unrelated individuals, 35 affected and 35 unaffected. The data was analyzed in its entirety and by examining the data by gender. We were able to identify a novel pathway for schizophrenia pathobiology. One of the pathways common among the three gene lists derived from this experiment is the estrogen signaling pathway. The estrogen receptor 1 (ESR1) and estrogen receptor 2 (ESR2) genes are up regulated 3 fold in schizophrenic males compared to unaffected males. We found no difference in either of these genes between the schizophrenic females and the unaffected females. However, we did find other genes in the estrogen signaling pathway and genes responsible for estrogen synthesis significantly up regulated in both male and female schizophrenics compared to the unaffected groups. The relative level of components of this pathway were examined at the protein level through immuno-histochemistry. The model developed from this research is able to account for a majority of the current literature surrounding schizophrenia including the many apparently divergent theories of schizophrenia etiology, genes known or thought to be associated with schizophrenia and the mode of action of antipsychotic medications prescribed for schizophrenia.

Disrupted-in-Schizophrenia-1 (DISC1) in Microtubular Dynamics and Cerebral Cortical Development. A. Kamiya^{1, 4}, T. Tomoda², K. Kubo³, R. Youn¹, Y. Ozeki^{1, 4}, P. Una⁵, M. Okawa⁴, C.A. Ross^{1, 5, 6}, M.E. Hatten², K. Nakajima^{3, 7}, A. Sawa^{1, 5}. 1) Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Laboratory of Developmental Neurobiology, The Rockefeller University, New York, NY; 3) Department of Anatomy, Keio University School of Medicine, Tokyo; 4) Department of Psychiatry, Shiga University of Medical Science, Otsu; 5) Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD; 6) Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD; 7) Department of Molecular Neurobiology, Institute of DNA Medicine, Jikei Univ. School of Medicine, Tokyo.

Although schizophrenia is one of the severest psychiatric illnesses, its pathophysiology remains unclear. Disrupted-in-Schizophrenia-1 (DISC1), identified at the breakpoint of a chromosome (1;11)(q42.1; q14.3) translocation tightly linked to schizophrenia in a Scottish pedigree, is also associated with schizophrenia in other populations, suggesting that it is a model for the pathogenesis of schizophrenia. The translocation causes C-terminal-truncation of DISC1 protein (mutDISC1). We show here that normal DISC1 is a component of the dynein/microtubule complex, regulating microtubular dynamics and neuronal migration. MutDISC1 acts as a dominant-negative, by binding to wild-type DISC1 and disturbing its normal intracellular distribution. Loss of DISC1 function impairs neurite outgrowth in vitro as well as neuronal migration in developing cerebral cortex in vivo. The defects induced by mutDISC1 support the idea that schizophrenia is a disorder of neurodevelopmental origin.

***ABCA4* mutations in recessive retinitis pigmentosa.** C.M. Zaremba¹, A.N. Yatsenko¹, W. Wiszniewski¹, M. Leppert⁵, R.A. Lewis^{1,2,3,4}, J.R. Lupski^{1,4}. 1) Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Dept. of Medicine, Baylor College of Medicine, Houston, TX; 3) Dept. of Ophthalmology, Baylor College of Medicine, Houston, TX; 4) Dept. of Pediatrics, Baylor College of Medicine, Houston, TX; 5) Dept. of Human Genetics, University of Utah, Salt Lake City, UT.

Retinitis Pigmentosa (RP) is a clinically and genetically heterogeneous group of inherited retinal disorders, which lead to a complete loss of vision. Among the known causative autosomal recessive (ar) RP genes, mutations in *ABCA4* (*ABCR*) hold interest for their high prevalence in the population. Genetic analyses of disorders associated with *ABCA4* alterations are complicated by the large size of the gene and the heterogeneity of its variants (> 400 known). To clarify the prevalence of *ABCA4* alterations in a cohort of 29 arRP families and to explore their role in the phenotype of the disorder, we used a commercial microarray, ABCR-400, in addition to direct sequencing and segregation analyses. In 7 out of 29 (~24%) arRP families we identified 9/58 (~16%) *ABCA4* alterations. Interestingly, in two arRP families, each of the *ABCA4* disease alleles cosegregated with RP (4/58, ~7%), whereas the other 5/58 (~9%) *ABCA4* mutations were heterozygotes. In one family we found a homozygous complex allele [L541P; A1038V], which segregated with the disease in two subjects with arRP. In vitro biochemical studies indicate that the mutant complex allele has a more severe effect on ATP hydrolytic activity (~30% of wild type) than each of the mutations alone by itself. In the second RP family we identified a severe truncating allele, R408X, with a missense alteration, R602W, which presumably has an effect on ABCR trafficking as determined by recombinant ABCR constructs expressed in *Xenopus laevis*. Segregation analyses in 5 arRP heterozygous families demonstrated no direct correlation between *ABCA4* alterations and the disease phenotype. We conclude that homozygous or compound homozygous *ABCA4* mutations account for ~7% of arRP and heterozygous *ABCA4* mutations, despite having functional consequences, do not specifically influence the RP phenotype.

Identification of pathways defective in MS: computer assisted integration of linkage and expression data. D.

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A total of 4 genome scans in populations of UK, US, Canada and Finland have identified a total of 20 chromosomal regions linked or suggestive for linkage to MS. Over 1,000 genes are detected in these 20 genomic regions, all representing potential candidate genes for MS. Similarly, 17 expression studies on MS using tissues ranging from peripheral blood mononuclear cells, CD4+ cells, MS plaques, human glioblastoma cells, oligodendroglial cells and tissues derived from rat MS model have identified tens of candidate genes. Combining data from expression and linkage studies could potentially provide exciting new avenues for MS research. However, this strategy is hampered by technical problems e.g. nomenclature of genes or gene markers are often not standardized among different studies. In addition, functional relationship between genes on critical chromosomal regions and differentially expressed genes are not established. We have designed a relational database containing candidate genes derived from all MS linkage and expression studies. A java front-end client was developed to allow users to query data from the gene database. To determine potential functional relationship between genes, we constructed a computational system to allow users to interrogate between multiple datasets. Currently, this computational system has been tested using three datasets: (1) the NCBI's 60 cell-line gene expression data; (2) a large human data sampled from a diverse array of tissues, organs, and cell lines (GNF gene expression Atlas); and (3) a mouse gene expression data from segregation experiments. The analyses provided include both correlation studies and liquid association (LA) study. LA analysis on MS loci on 5p13 and 17q22-24 suggest a functional relationship between FALZ and SLC1A3 genes exemplifying identification of novel targets for further studies.

Computational strategies to identify candidate genes for genetically complex diseases: type 2 diabetes mellitus.

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Complex diseases involve an interaction of genetic and environmental components in disease susceptibility, suggesting a search for candidate genes that show both genetic variability and environmental response in predisposing disease. Genetic linkage analysis localizes candidate genes that may have allelic variation related to disease, while differential expression analysis reveals genes that show an important cellular response to the environment. Using Type 2 Diabetes Mellitus (T2DM) as a test case, we show that combining genetic linkage and differential gene expression criteria yields better candidate genes than selection by either criterion alone. T2DM is used as a test case to validate the method because of its significant public health impact and complex disease susceptibility, as well as the availability of data from previous research, which provides input for the method. Using criteria developed during testing, we identified four candidate T2DM genes, which are linked to T2DM and also show differential expression coincident with the onset of disease. Among these candidates, two were previously implicated in T2DM (PTPN1 and SCARB1) and two are novel (CAPN6 and DHRS8). Notably, CAPN6 is a member of the calpain family, which is implicated in T2DM, and CAPN6 is structurally similar to CAPN10 (a.k.a NIDDM1). These four candidates also share a common element in retinol (Vitamin A) metabolism, which has been associated with T2DM susceptibility. Approximately 1.6 % of the genes assayed were previously implicated in T2DM, yielding a probability of 0.0015 for finding two or more T2DM genes in a random selection of four, suggesting that the two genes not previously implicated in T2DM are also valid candidates. More generally, these criteria are effective in improving the selection of candidate genes for one complex disease, and this method could be applied in identifying candidate genes for other complex diseases.

Gem GTPase and tau: morphological changes induced by Gem GTPase in CHO cells are antagonized by tau. F.

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A series of observations have indicated that tau, one of the major microtubule-associated proteins, is involved in neuronal cell morphogenesis and axonal maintenance. Tau is also the major component of paired helical filaments found in the brain affected by Alzheimers disease. To explore an as yet unidentified role of tau in vivo, ~11,000 mRNAs were profiled from tau-deficient mouse brains, and compared with those from control brains at the same ages. The expression of Gem GTPase, a small GTP-binding protein of the ras superfamily, was significantly increased in the brain of tau-deficient mice at eight weeks of age. Because Gem GTPase is a negative regulator of the Rho-Rho kinase pathway for cytoskeletal organization, this protein was transiently overexpressed in CHO cells that do not express tau. Overexpression of Gem GTPase induced a marked elongation of CHO cells, and simultaneous expression of tau eliminated this effect, although tau did not bind directly to Gem GTPase. This anti-elongation activity of tau was attributed to its microtubule (MT)-binding domain, and homologous domains of MAP2 and MAP4 exhibited similar antagonistic activities. Taken together, the present results indicate that the level of Gem GTPase and its cell elongation activity are modulated by tau, and suggest that tau may be involved in a Gem GTPase-mediated signal transduction pathway.

Mitochondrial Complex I Nuclear Genes and Parkinsons Disease. *M.D. Ritchie¹, S.L. Lee², P. Silburn³, J. Prince⁴, A. Brookes⁴, G.D. Mellick⁵*. 1) Center for Human Genetics Research, Vanderbilt Univ, Nashville, TN; 2) Dept of Neurology, Vanderbilt Univ, Nashville, TN; 3) Dept of Neurology, Princess Alexandra Hospital, Brisbane, Australia; 4) Dept of Genomics and Bioinformatics, Karolinska Institute, Stockholm, Sweden; 5) Univ of Queensland, School of Medicine.

Identifying susceptibility genes for common, complex disease is at the forefront of genetic epidemiology. Parkinsons disease (PD) is among the debilitating neurological disorders that have become a major focus of human genetics. Genes involved in oxidative stress and mitochondrial activity have been suggested candidate genes for PD. We reason that genetic variations in mitochondrial Complex I nuclear genes may contribute in a complex manner to disease risk. In an initial screen of 306 PD patients and 321 unrelated healthy controls ascertained from a Caucasian Australian population, no association was observed between 70 SNPs in 31 mitochondrial Complex I nuclear genes and disease risk. In this study, we used a novel statistical approach, Multifactor Dimensionality Reduction (MDR) to analyze this data along with pesticide exposure history obtained by self-reported questionnaire to explore the possibility of gene-gene and gene-environment interactions with respect to mitochondrial complex I polymorphisms and pesticide exposure. MDR was developed as a nonparametric, model free method to test for association in case-control studies of human disease. Using MDR, we detected a single locus effect in *NDUFS4* (SNP00005133, HGVbase ID) which is able to correctly predict disease status with 56.22% accuracy ($P < 0.162$). When stratified by gender, MDR also detected a highly significant association in women between the dihydrolipoyl succinyl transferase gene (*DLST*) (SNP00000234) and disease status, 61.36% prediction accuracy ($P < 0.014$). Interestingly no high-order interactions were detected between these polymorphisms and the pesticide exposure data. These findings support the possibility that *NDUFS4* is an important factor in PD susceptibility among Caucasian individuals, and that *DLST* may contribute to disease risk in women and demonstrate the utility of novel computational approaches for the detection of disease susceptibility genes.

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Ethanol responsive metabolome: An insight in the regulatory network involving trans-acting factors and cis-acting motifs. *R.K. Uddin, J.A. Treadwell, S.M. Singh.* Molecular Genetics Unit, Department of Biology and Division of Medical Genetics, University of Western Ontario, London, Ontario, Canada N6A 5B7.

The effect of alcohol is realized by its effect on cellular physiology via gene expression. Ultimately it mediates alcohol's effect in the short as well as in the long term, resulting in drunkenness, FAS and addiction. It is now apparent from the genome wide works on the effects of alcohol on gene expression that some genes are invariably up-regulated or down-regulated while others are affected in an individual specific manner, depending on the genotype, age, dose or other factors. The genes identified fall in such functional categories as, cell signaling, gene regulation, stress response, protein modification, proliferation/survival and transport (Treadwell and Singh, 2004). Further a high proportion of these genes, map to QTL regions for various alcohol specific phenotypes. This comprehensive list of genes (~100) requires a more interactive and metabolomic approach for its interpretation, where a chain reaction regulates expression of a set of genes that involves specific cis-element(s) and trans-acting factor(s) and ultimately yielding a complex network of expression. An understanding of this network is vital to the elucidation of the alcohol responses and effects. We have undertaken an exhaustive knowledge based analysis of the 5' upstream sequence of the ethanol responsive genes identified by microarray to (predict and) compile a searchable database of Ethanol- Responsive-gene-Regulatory-Network (EthaRNet) involving cis-motifs and their interacting trans-factors. Individual genes and their regulatory elements implicated in alcoholism in the literature are also included in the analysis. We generate a number of individual regulatory motifs - protein interaction processes for all genes and map them in networking pathways that provides a global view of ethanol responsive transcriptional / post-transcriptional regulatory networks. The pathways are connected and allow us to predict a list of cis-regulatory elements active in the pathways that are specific to ethanol responses and effects. Supported by Canadian Institute of Health Research and Ontario Mental Health Foundation.

Quantitative TDT analysis of SNPs of candidate genes for bone mineral density in 1p36. *M. Devoto¹, K. Sol-Chuch¹, D. Stabley¹, H. Li¹, C. McKay¹, A. Tenenhouse², L.D. Spotila³.* 1) Nemours Children's Clinic, Wilmington, DE; 2) McGill University, Montreal, ON, Canada; 3) Drexel University, Philadelphia, PA.

Low bone mineral density (BMD) is one of the most important risk factors for osteoporosis, a common complex trait characterized by reduced skeletal strength and increased susceptibility to fracture. We performed a whole genome scan in Caucasian families of individuals with low BMD and identified a candidate region on 1p36 by variance component linkage analysis (maximum multipoint lod-score of 3.53 for linkage of femoral neck BMD near marker D1S214) and quantitative transmission disequilibrium test (QTDT) ($p = 0.0098$ for marker D1S2660, and $p = 0.0012$ for D1S489). To follow up on these findings, we have selected a few candidate genes on the basis of their location in 1p36 and information about function and pattern of expression. SNPs of these genes have been tested for linkage disequilibrium to femoral neck BMD in the same sample of families. Genotyping of SNPs obtained from publicly available databases or from Applied Biosystems Assays-on-Demand was performed by either Pyrosequencing or on the ABI 7900HT Sequence Detection System. Linkage disequilibrium of candidate gene SNPs and femoral neck BMD in the 40 families was tested by means of the QTDT software including sex, age and BMI as covariates. Results of this analysis were not significant for nine SNPs of the guanine nucleotide binding protein (G protein) beta polypeptide 1 gene (GNB1) and 10 SNPs of the calmodulin binding transcription activator 1 (CAMTA1) (all p -values > 0.1). One SNP of four tested in the isoprenylcysteine carboxyl methyltransferase gene (ICMT) yielded a p -value of 0.045. A fourth gene, WD repeat-containing protein 8 (WDR8) was selected because experiments in mouse had shown expression in bone and cartilage and in bone forming cells including osteoblasts and chondrocytes during ossification. Among six SNPs tested in this gene, one yielded a nominal p -value of $p = 0.005$ for association with femoral neck BMD. Additional tests are in progress to confirm these finding using additional SNPs of the same genes and haplotype analysis.

Association of single nucleotide polymorphisms (SNPs) in the PPARG gene and interaction with COL1A1 gene in susceptibility for osteoporosis in elderly women. *J. Li¹, S.K. Ro¹, N. Umblas¹, J. Mangacat¹, L. Lui², J.M. Zmuda³, S.R. Cummings⁴, G. Peltz⁵, R. Higuchi¹.* 1) Roche Molecular Systems, Alameda, CA; 2) University of California, San Francisco, CA; 3) University of Pittsburgh, Pittsburgh, PA; 4) California Pacific Medical Center, San Francisco, CA; 5) Roche Bioscience, Palo Alto, CA.

PPARG has been shown in a mouse model to be involved in osteogenesis. A silent mutation in exon 6 of the PPARG gene and a mutation in an Sp1 binding site in intron 1 of the COL1A1 gene have been reported to be associated with reduced bone mineral density (BMD) in humans. We conducted a nested case-control osteoporosis (OP) study (n=1042) among older women that involved genotyping of 23 SNPs in 6 genes, including 6 SNPs in PPARG and the Sp1 polymorphism in COL1A1. PPARG and COL1A1 allele and genotype frequencies in women with incident hip (n=275) and vertebral (n=262) fractures (HFX and VFX), and low BMD (n=276) were compared with those in controls without these phenotypes (n=278). Two of the PPARG SNPs (Pro12Ala and VN102), which are in linkage disequilibrium, showed an association with HFX. Individuals with HFX have 7.0 and 7.7% higher frequency of the genotype CC (Pro12Ala) (odds ratio [OR]=1.8, p=0.02 after adjusting for age, weight, and estrogen use [adjustment]) and GG (VN102) (OR=1.9, p=0.01 after adjustment) than those in controls, respectively. There was no significant association between PPARG and VFX and low BMD after adjustment. However, we observed an interactive effect between PPARG and COL1A1 genotypes on both VFX and low BMD (p=0.009 and 0.002 respectively after adjustment). For VFX and low BMD, the OR for the Pro/Pro genotype among women who had the COL1A1 risk allele was 2.9 (p=0.01) and 5.9 (p=0.0008) respectively after adjustment. In contrast, there was no significant relationship between PPARG Pro/Pro genotypes and any of above three OP phenotypes among women who had no COL1A1 risk allele. The same effect was also observed for PPARG_VN102. These data indicate a novel association between PPARG and HFX and an interlocus interaction between PPARG and COL1A1 and the genetic susceptibility to OP in older Caucasian women.

Absence of association of the matrilin-3 polymorphism T303M (rs8176069) with osteoarthritis. *J.L. Min¹, I. Meulenbelt¹, N. Riyazi², M. Kloppenburg², H.A. Pols³, C.M. van Duijn⁴, P.E. Slagboom¹.* 1) Medical Statistics, LUMC, Leiden; 2) Rheumatology, LUMC, Leiden; 3) Internal Medicine, Erasmus MC, Rotterdam; 4) Epidemiology & Biostatistics, Erasmus MC, Rotterdam, The Netherlands.

Osteoarthritis (OA) is a joint disease characterized by degeneration of articular cartilage and remodeling of the subchondral bone with sclerosis. It has been demonstrated that genetic factors play a considerable role in the onset of OA described by various definitions. *MATN3* is an interesting candidate gene for OA encoding a noncollagenous extracellular oligomeric matrix protein. In this gene, an association of a missense mutation (T303M) involving a conserved amino acid substitution among patients with hand OA (0.02) as compared to the Icelandic population (0.01) was observed. To replicate these findings, we have genotyped three polymorphisms, rs2242190, rs8176069 (T303M) and rs8176070 in the *MATN3* gene in two populations. Firstly, a random sample of 809 subjects from a Dutch population-based cohort (the Rotterdam study) was studied. In this study, radiographic OA for hip, knee, hand and spine OA was assessed. Secondly, the GARP study (Genetics, Osteoarthritis and Progression) was investigated consisting of 191 affected siblings with symptomatic and radiographic OA in more than one joint group. Both in the Rotterdam (0.014) and the GARP study (0.011), we observed a 2-3 times higher allele frequency of T303M than in the Icelandic population (0.005). In contrast to the Icelandic study, no significant association with similar phenotypes for hand OA in the Rotterdam study was observed. In addition to what was studied in the Icelandic population, we tested whether the T303M variant and/or haplotypes of the three *MATN3* polymorphisms were associated with OA of the hip, knee or spine. Among 21 T303M carriers, OA was not occurring more frequently than in the 809 subjects from the Rotterdam study. The three polymorphisms combined to major haplotypes of which the frequencies were similar to that in the Icelandic population. None of these haplotypes were consistently associated with the presence of OA at any joint group tested. Our results do not support the T303M variant to be involved in OA.

Association between a *CCR5* haplotype and juvenile rheumatoid arthritis. *S. Prahalad*¹, *J.F. Bohnsack*¹, *D. Dunn*², *S.D. Thompson*³, *D.N. Glass*³, *R. Weiss*², *M. Bamshad*^{1,2}. 1) Dept Pediatrics, Univ Utah, Salt Lake City, UT; 2) Dept Genetics, Univ Utah, Salt Lake City, UT; 3) Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Juvenile rheumatoid arthritis (JRA), the most common cause of chronic childhood arthritis, is mediated by Th1-immune responses. In children with JRA, synovial fluid T-cells express high levels of the Th1-chemokine receptor *CCR5*, which has been implicated in susceptibility to rheumatoid arthritis. We hypothesized that genetic variation in the 5'*cis*-regulatory region of *CCR5*, which are known to cause variable *CCR5* expression, will be associated with susceptibility to JRA. To test this hypothesis, we analyzed patterns of variation in the 5'*cis*-regulatory region of *CCR5* in a well-characterized cohort of 124 multiplex families containing 250 affected children from a NIH-sponsored JRA affected sibpair registry. The upstream region of human *CCR5* (-2867 to -1745), which included the *CCR5* promoter, was sequenced using standard methods. Ten single nucleotide polymorphisms (SNPs) and 8 haplotypes were identified, most of which have been described previously. SNPs and haplotypes were tested for association with JRA by transmission disequilibrium testing (TDT) using Transmit 2.5.4. Analyses were repeated after stratification by onset-type and age of onset. Two common haplotypes were observed. TDT did not demonstrate deviations of expected transmission of any of the *CCR5* SNPs or haplotypes to the JRA cohort as a whole. However, after stratification, one of the *CCR5*-haplotypes was significantly ($p < 0.04$) under-transmitted to children with JRA who had disease onset before age 6. The same haplotype also was under-transmitted to children with polyarticular JRA, but this did not reach statistical significance ($p = 0.08$). The SNP (C927T) that defined this haplotype was also under-transmitted to children with early onset JRA ($p = 0.053$). Interestingly the presence of this *CCR5*-haplotype has been previously shown to influence the progression of acquired immune deficiency syndrome. We conclude that genetic variants in the *cis*-regulatory region of *CCR5* might be associated with early onset juvenile rheumatoid arthritis.

Association of a novel serotonin transporter polymorphism with obsessive-compulsive disorder based on qualitative and quantitative traits. *P.D. Arnold¹, X. Hu², J. Trakalo¹, M.A. Richter¹, D. Goldman², J.L. Kennedy¹.* 1) Centre for Addiction and Mental Health, Toronto, ON, Canada; 2) NIAAA/NIH, Rockville, MD, USA.

There is strong evidence that genetic determinants are involved in the etiology of Obsessive-Compulsive Disorder (OCD). Previous candidate gene investigations have indicated a possible association with the high transcribing long (L) allele of the serotonin transporter (5HTT) gene-linked polymorphic region (5HTTLPR), however findings have been inconsistent. Earlier studies have not accounted for a recently identified A/G variant within the L allele which alters transcriptional activity, with the more common LA variant associated with increased transcription and the LG allele associated with decreased transcription. In a recent case-control study, the LA allele was associated with OCD (Goldman et al., 2004). We genotyped the 5HTTLPR and the A/G variant within the 5HTTLPR in 172 nuclear families ascertained through an OCD proband, and obtained data regarding symptom severity in 139 of the probands and 36 siblings using the Yale-Brown Obsessive Compulsive Scale (YBOCS). Analysis for genetic association was conducted using the Family Based Association Test (FBAT), based on both qualitative (OCD diagnosis) and quantitative (highest known YBOCS score) traits under additive, dominant and recessive models of inheritance. For OCD diagnosis, a nominally significant association was found with the LA allele under the dominant model of inheritance ($z=2.01$, $p=0.04$). For symptom severity, the strongest finding was again for the dominant model, with a positive association with the high transcribing LA allele ($z=2.67$, $p=0.007$) that remained significant after correcting for multiple models of inheritance. These results indicate that the high transcribing LA allele may influence the OCD phenotype through its effects on expression of the serotonin transporter, and replicate the findings of an independent case-control sample. Furthermore, the stronger results based on symptom severity underscore the usefulness of analyzing quantitative traits in complex neuropsychiatric disorders.

Multi-locus effects with RELN, APOE, and SLC6A4 influence autistic disorder susceptibility. *A.E. Ashley-Koch¹, J. Jaworski¹, H. Mei¹, R. Rabionet¹, D.A. Skaar¹, M.D. Ritchie², G.R. DeLong¹, R.K. Abramson³, H.H. Wright³, M.L. Cuccaro¹, J.G. Gilbert¹, E.R. Martin¹, M.A. Pericak-Vance¹.* 1) Duke University Medical Center, Durham, NC; 2) Vanderbilt University, Nashville, TN; 3) University of South Carolina School of Medicine, Columbia, SC.

Autistic disorder (AD) is a complex genetic disorder with several putative susceptibility loci. We have examined three candidate genes (RELN, APOE and SLC6A4) to test whether they jointly confer AD risk. RELN is a positional, functional candidate gene for a 7q AD locus. Although AD linkage has not been observed in the APOE region of chromosome 19, RELN binds two APOE receptors suggesting these two genes may interact. SLC6A4 is involved in regulation of serotonin, a biomarker for AD, and several studies have reported association between SLC6A4 and AD. We examined these genes in 241 multiplex (including 99 from AGRE) and 210 singleton AD families. All AD individuals met ADI-R criteria. All SNPs examined were tested for Hardy-Weinberg Equilibrium deviations and for LD with other SNPs. We used the geno-PDT, to detect joint multi-locus associations, and the multifactorial dimensionality reduction (MDR) method. Our first hypothesis was that RELN interacts with APOE. In the overall dataset, both the geno-PDT and MDR detected significant ($p < 0.05$) 2-locus effects. However, the most significant joint effects were detected in the multiplex subset where the geno-PDT gave a significant global result ($p = 0.001$) for a SNP at position 113 in APOE with a SNP in exon 59 of RELN. The MDR analysis also detected a significant 2-locus effect between these SNPs ($p = 0.02$). The next hypothesis tested was whether RELN interacts with SLC6A4. In the singleton subset, there was evidence for a multi-locus effect of several SNPs involving combinations of 2 and 3 SNPs from both genes in the MDR analysis ($p < 0.001$). This effect was supported by MDR analysis of the entire dataset ($p = 0.02$), as well as by geno-PDT analysis ($p < 0.001$). We are further exploring these multi-locus effects to disentangle main effects and interactions. In summary, these preliminary results suggest that multi-locus effect do indeed contribute to AD susceptibility.

Collecting phenotypic data for gene discovery in Parkinsonism-the NINDS cell and DNA repository. *J. Beck¹, J. Nash¹, C. Royds¹, C. Kronenthal¹, R. Malkani², J. Werner², A. Crawley², D. Murphy², B. Ravina², K. Gwinn-Hardy².*
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While biological repositories exist for genetic studies in a variety of disorders, it is rare to find those which have standard clinical data sets and are widely available as public resources. To develop a large well-characterized sample collection in neurological disorders, the National Institute of Neurological Disorders and Stroke (NINDS) Cell line and DNA repository (<http://locus.umdj.edu/ninds>) developed clinical assessment tools for use in genetic studies of Parkinsons disease (PD) and other forms of parkinsonism. Also included in the collection are affected and unaffected blood relatives, spousal controls, and normal healthy individuals. Clinical Data Elements (CDEs) were developed which allows a standard set of phenotypic information to accompany each sample based on clinical signs. The PD CDEs were designed to reflect the widely utilized London Brain Bank Criteria in a format designed for easy use in the clinic or field. Additional design was based on the results of an NINDS sponsored meeting. Most patients with parkinsonism have PD. However, parkinsonian signs may be present in neurodegenerative disorders with different neuropathological and genetic characteristics; these include Progressive Supranuclear Palsy (PSP), Multiple System Atrophy (MSA), Corticobasal Degeneration (CBD), Spinocerebellar Ataxia (SCA) and others. Submitters obtain clinical data from the subjects history and physical examination, submit these with each sample, and these are reviewed by a board certified neurologist to ensure appropriateness for inclusion in the repository. CDEs for Parkinsonism focus on PD, but include elements which address features suggestive of other diagnoses. Additionally, separate CDEs for non-PD parkinsonian subjects, and for controls, have been developed. Electronic formats have also been developed for housing and manipulating these data, allowing simple searches for a given endophenotype, and focused genotype-phenotype studies. These clinical and databasing tools permit pooling of samples and clinical information across many studies.

***SYBL1* as a candidate gene for autism, a mixed genetic-epigenetic model.** S. Ben Shachar¹, M. Shinawi¹, Y. Jiang¹, R.E. Stevenson², A.L. Beaudet¹. 1) Molecular & Human Genetics, Baylor Col of Medicine, Houston, TX; 2) Greenwood Genetic Center, Greenwood, SC.

Autism is a complex psychiatric condition with high male to female ratio. Significant genetic contribution to autism can be implied by its high concordance rate in monozygotic twins. So far genome-wide linkage studies have failed to provide strong evidence for major autism-related loci suggesting existence of many loci. Alternatively, epigenetic changes might contribute to its etiology. *SYBL1* encodes a member of the synaptobrevins family of proteins that are involved in synaptic vesicle docking and exocytosis. It is ubiquitously expressed in many tissues including many brain structures. Interestingly, *SYBL1* is located on Xq and Yq pseudoautosomal region. It is unique in being subject to Y-specific inactivation in males. The inactivation is associated with CpG methylation of the gene promoter. We hypothesized that aberrant expression of *SYBL1* might cause autism. Defects might be de novo or inherited, ubiquitous or brain-specific, and might be associated with aberrant DNA methylation. This hypothesis could explain both the failure to find a major autistic gene and the male predominance in autism. We analyzed the methylation of the promoter region of *SYBL1* in lymphoblast cell lines of 29 autistic individuals from AGRE and NIMH collections (families with affected sib pairs), 10 from S. Carolina Autism Project (families with a single affected individual), and 24 controls. Furthermore, 19 autistic brains were examined. The methylation status was tested by southern blot analysis after double digestion with *EcoRI* and the methylation sensitive enzyme *BssH II*. Furthermore, DNA was sequenced after bisulfite treatment to precisely evaluate the CpGs' methylation of this region. The expected 1:1 ratio of methylated:non methylated allele was found in all of the brain DNAs. In the autistic lymphoblast cell lines, 3 showed hypermethylation, and 2 demonstrated hypomethylation. Among controls, we found 1 cell line with hypermethylation and 1 with hypomethylation. We conclude that alteration of expression of *SYBL1* as tested by methylation doesn't have a major contribution to the autistic phenotype.

The relationship of the 5HTTLPR polymorphism to serotonin transporter mRNA transcription. *S.L. Bradley, K. Dodelzon, H.K. Sandhu, R.A. Philibert.* Psychiatry, University of Iowa, Iowa City, IA.

The serotonin transporter (5HTT; chromosomal location 17q12) is an important regulator of monoaminergic neurotransmission and is the site of action for a number of antidepressant medications including fluoxetine (Prozac) and sertraline (Zoloft). Not surprisingly, sequence variation at 5HTT has been associated with a wide variety of neurobehavioral illness. In particular, variation at a polymorphic site known as the 5HTTLPR, which is 1.4 kb upstream of the translation start of 5HTT, has been associated in some studies with increased vulnerability to depression, neuroticism, and autism. Support for these clinical observations has included laboratory findings that 5HTTLPR variation is associated with large changes in 5HTT gene translation in a small sampling of lymphocyte cell lines (n=11). We re-examined these earlier findings by directly measuring genotype and 5HTT mRNA levels in over 80 independent lymphocyte cell lines using PCR and RT-PCR methodologies. The resulting data were analyzed by ANOVA. We found a trend for a relationship between 5HTTLPR genotype and mRNA levels with the genotype accounting for less than 8% of the total variance in 5HTT mRNA production. Furthermore, in contrast to previous reports, the small effect of 5HTTLPR variation on 5HTT mRNA production appeared to be mediated through an additive, not dominant, mechanism. These results and additional data are discussed. We conclude that although there are a number of possible explanations for our results, we conclude that further examination of the relationship of the role of the 5HTTLPR in regulating 5HTT mRNA production is warranted.

Single Nucleotide Polymorphism Analysis on Chromosome 2 in Early-Onset Alzheimer Disease Families. *C.A. Browning¹, S. Prasad¹, P. Bronson¹, D.D. Vance¹, J.M. van der Walt¹, W.K. Scott¹, J.L. Haines², M.A. Pericak-Vance¹.*
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Recent analysis of 10cM whole genomic screen microsatellite data with age at onset as a covariate identified a region of chromosome 2 linked (LOD score = 3.2) in families with average onset of Alzheimer disease (AD) in the fifth and sixth decades. Twelve single nucleotide polymorphisms (SNPs) spanning 2.5 Mb and flanking the peak marker (D2S2944) were genotyped in 31 families (with an average of three sampled affected individuals/family) with at least one family member with early-onset AD (50-60 years). SNPs were identified through ABI Assay on Demand and genotyped using the TaqMan system. Non-parametric linkage analysis was performed using Allegro. The multipoint LOD score increased to 3.5 and peaked at rs1370369 (single point LOD score = 1.48, multipoint LOD score = 3.46). The -1 lod interval spans ~ 17 Mb. These data increase support for a locus on chromosome 2 restricted to a subset of families with early-onset family members. Mutation analysis is ongoing for functional candidate genes mapping to 1-LOD unit down support interval.

No evidence of linkage disequilibrium between RGS4 and schizophrenia in Canadian linkage families. *L.M.*

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We have previously reported linkage of schizophrenia to chromosome 1q21-22 with a heterogeneity lod (HLOD) score of 6.50 in a group of 22 medium-sized multiplex Canadian families. Most recently, we have reported significant evidence of linkage disequilibrium (LD) between schizophrenia and markers within the gene CAPON on 1q22. Other groups have reported significant association between schizophrenia and RGS4, a gene located 700 kb distal to CAPON on 1q23. We evaluated RGS4 for LD to schizophrenia in our sample, as a potential second susceptibility locus on 1q.

To identify SNPs for LD analysis, we resequenced the RGS4 gene in 16 subjects from our sample, including the entire coding region of the gene, introns 2-4, and 1 kb of 5' region, resulting in the identification of 14 SNPs. No SNPs within coding sequence or splice sites were identified. For LD analysis, we genotyped our entire current sample of 332 subjects for 4 SNPs previously reported to be associated with schizophrenia, and another SNP just 5' to the promoter and one in the 3' UTR. Evidence for LD was assessed using the program PSEUDOMARKER which, in contrast to popular TDT-based programs, explicitly separates the evidence for linkage and LD in families with multiple affecteds. Analyses were conducted using a recessive pseudomarker model, as there is significantly stronger evidence for a recessive mode of action for the 1q locus in this sample, and using a narrow definition of affection (schizophrenia or schizoaffective disorder). No marker produced significant ($p < 0.05$) evidence for LD to markers in RGS4. This same sample and same analysis method had previously produced significant evidence (empiric study-wide p -value < 0.05) of LD to 3 markers in CAPON. These results fail to provide evidence for RGS4 as a second 1q schizophrenia susceptibility gene in this sample.

A mutation in the *ATPIA2* gene in a large family with migraine without aura. *M. Castro*^{1,2}, *C. Lemos*^{1,2}, *J. Barros*³, *C. Fraga*³, *L. Guimaraes*⁴, *P. Maciel*^{2,5}, *A. Sousa*^{1,2}, *J. Sequeiros*^{1,2}, *J. Pereira-Monteiro*^{1,2,3}, *I. Silveira*^{1,2}. 1) IBMC, UnIGENE, Porto, Portugal; 2) ICBAS, Universidade do Porto; 3) Serviço de Neurologia, HGSA; 4) Faculdade Ciências Saúde, Universidade Fernando Pessoa;; 5) ICVS, Escola Ciências Saúde, Univ. Minho, Braga, Portugal.

Migraine is a common, complex and heterogeneous disorder, presenting strong familial aggregation. Recently, the gene encoding the Na⁺/K⁺ pump 2 subunit (*ATPIA2*) has been implicated in a rare and severe familial type of migraine with hemiparesis. We selected 50 families with an apparently autosomal dominant mode of inheritance of migraine without aura (MO) or with aura (MA) and performed mutation analysis to find out whether this gene was also involved in the common type of migraine. A G-to-A transition was found at nucleotide 256, in exon three, in a large family with MO. This mutation causes an arginine-to-histidine change at codon 51 of the Na⁺/K⁺ pump 2 subunit, and was not present in 346 chromosomes from a control population. This Arg51 is conserved among subunits of the pump from other species. Na⁺/K⁺-ATPase exchanges intracellular Na⁺ for extracellular K⁺, generating ion gradients important for nerve transmission and muscle contraction. This R51H change is located in the N-terminal cytoplasmic loop, in a predicted cation ATPase transporter domain. No mutations were found in the remaining families. In conclusion, we describe the first mutation in a large family with MO. These results also indicate that the common type of migraine may in fact be caused by impaired ion homeostasis in brain, at least in some families.

Microrrearrangements of human chromosome 15q11-q13 in families with autistic disorder. *C. CERRATO*¹, *M. Ogorelkova*¹, *M. Gratacòs*¹, *X. Estivill*^{1,2}. 1) GENES AND DISEASES, CENTER FOR GENOMIC REGULATION, BARCELONA, BARCELONA, Spain; 2) Experimental and Health Sciences Department, Pompeu Fabra University, Barcelona Biomedical Research Park, Barcelona, Catalonia, Spain.

Autism is a neurodevelopmental disorder exhibiting complex genetic etiology. Human chromosome 15q11-q13 is a candidate region to autism based on linkage, linkage disequilibrium, cytogenetic abnormalities, and maternal duplications at this locus in patients with autism and autism-related phenotype. Chromosome 15q11-q13 contains several imprinted genes expressed in the brain, including small nucleolar RNA (snoRNA) genes, and is rich in segmental duplications. These sequences promote fairly frequent 15q11-q13 rearrangements resulting in Prader-Willi syndrome, Angelman syndrome, mental retardation and autism spectrum disorder. Among plausible candidate genes for autism in this region is cluster of the gamma-aminobutyric acid (GABA) receptor genes. In order to detect possible duplicon-mediated submicroscopic rearrangements of 15q11-q13, we have performed the analysis of microsatellite markers in autistic families and control samples. We have found maternally and paternally inherited duplications of microsatellite located at HBII-85 snoRNA locus in two patients, and paternal deletion at microsatellites of the GABA region in one patient. Using quantitative PCR, PFGE and additional microsatellites and single nucleotide polymorphisms in the region we are currently defining the size of these rearrangements, which should help to delineate the relationship between 15q11-q13 microrrearrangements and autistic disorder.

Supportive evidence for a relationship between genetic variants at the *brain-derived neurotrophic factor (BDNF)* locus and depressive symptoms in affective disorder and schizophrenia. S. Cichon¹, J. Schumacher², R. Abou Jamra², T. Becker³, S. Ohlraun⁴, N. Klopp^{5,6}, E. Binder⁷, T.G. Schulze⁴, W. Maier⁸, A. Van Den Bogaert⁹, T. Illig^{5,6}, P. Propping², F. Holsboer⁷, M. Rietschel^{4,8}, M.M. Nöthen^{1,2}. 1) Life & Brain Center, Univ Bonn, Bonn, Germany; 2) Inst Human Genetics, Univ Bonn, Bonn; 3) Inst Medical Biometry, Informatics and Epidemiology, Univ Bonn, Bonn; 4) Central Inst Mental Health, Division of Genetic Epidemiol in Psychiatry, Mannheim, Germany; 5) Genome Analysis Center, GSF-National Research Center for Environment and Health, Neuherberg, Germany; 6) Inst Epidemiology, GSF, Neuherberg, Germany; 7) Max-Planck-Inst of Psychiatry (MPI), München, Germany; 8) Dept Psychiatry, Univ Bonn, Bonn; 9) Dept Molecular Genetics, VIB8, Univ Antwerp, Antwerp, Belgium.

There is evidence that the brain derived neurotrophic factor (BDNF) is involved in the pathogenesis of neuropsychiatric disorders and in the therapeutic action of at least some effective drugs. The aim of this study was to investigate the potential influence of genetic variation at the BDNF locus on the development of major depressive disorder (MDD), bipolar affective disorder (BPAD), and schizophrenia. We genotyped three polymorphisms at BDNF (rs6265 leading to Val66Met, rs988748, and a (GT)_n repeat) in 465 MDD patients, 533 schizophrenia patients, 281 BPAD patients, and 1,097 controls of German origin (Bonn sample). A second sample (312 MDD patients, 444 controls) was recruited in Bavaria (Munich sample). Three-marker haplotype rs988748-(GT)_n-rs6265 produced significant associations with all phenotypes under investigation ($p=0.00006$ for Bonn MDD, $p=0.0092$ for Munich MDD, $p=0.0057$ for BPAD, $p=0.016$ for schizophrenia). Stratification of the schizophrenia sample according to the presence or absence of comorbid mood or depressive disorder showed much smaller p-values for schizophrenia with comorbid depression ($p=0.0011$) in comparison with the complete sample. In conclusion, we find supportive evidence for a relationship between genetic variants at the BDNF locus and depressive symptoms in affective disorder and schizophrenia.

FRAXE intermediate alleles are associated with Parkinson's disease. *I.C. Ciro' Candiano¹, P. Tarantino¹, N. Cutuli², G. Milana², F. Annesi¹, E.V. De Marco¹, F. Condino¹, D. Civitelli¹, P. Spadafora¹, G. De Cicco¹, S. Carrideo¹, G. Annesi¹.* 1) Inst of Neurological Sciences, National Research Council, Mangone, Cosenza, Italy; 2) Laboratory of Molecular Genetics, Dept of Services, University of Catania, Italy.

The aim of the present study was to analyze the FRAXA and FRAXE triplet repeat variation and relative allele frequency in a population of male subjects with Parkinson's disease in comparison with normal controls. A total of 203 male patients with idiopathic PD from southern Italy were included in the study. Controls were 370 male healthy subjects from southern Italy enrolled during a previous study on aging. To determine trinucleotide repeat sizes, amplification of genomic DNA was performed using the polymerase chain reaction (PCR). FRAXA repeat sizes were determined using primers FXD and FXE. In a separate reaction, FRAXE repeat sizes were determined using primers 598 and 603. Overall allele distributions for FRAXA and FRAXE in normal controls were similar to those found in other studies. We did not find any cases of FRAXA full mutation or premutation in any of the 203 male subjects with PD or the 370 healthy controls. No difference was found in the allele distribution between patients and controls for FRAXA. No cases of FRAXE full mutation or premutation were found in either the patients or the controls. The distribution of allele size for FRAXE was significantly different (Chi squared=6.30; $p < 0.04$) between patients and control. Moreover, when the FRAXE intermediate alleles (31-60 repeats) were considered, there was a significant excess of these alleles among the experimental chromosomes ($p < 0.001$). Indeed, 13 out of 203 subjects with PD (6.4%) carried FRAXE intermediate alleles ranging from 31 to 54 repeats in respect to only one out of 370 normal controls who carried an allele of 37 repeats (0.27%). Thus, subjects carrying FRAXE intermediate alleles have a significantly increased risk of developing PD (OR 95% CI, 25.25; 3.3-194.4). The present study demonstrates that intermediate alleles for FRAXE are over-represented in male subjects with PD in respect to normal control.

Mutation Screening of NLGN3 and NLGN4 in Autism Spectrum Disorders. *L.N. Clark¹, S.K. Afridi¹, B. Chih², P. Scheiffele².* 1) Dept Pathology & Taub Inst, Columbia Univ, New York, NY; 2) Dept of Physiology and Cellular Biophysics, Center for Neurobiology and Behavior, Columbia University New York, NY.

Autism-spectrum disorders (ASD) are a heterogeneous group that differ in severity and age at onset but can share one or more of the clinical features that includes impairment in social interaction and communication, repetitive and/or obsessive-compulsive behaviors, and mental retardation. There is strong evidence for a genetic involvement in the etiology of ASD. Recent studies have implicated a family of neuronal cell adhesion molecules called Neuroligins (NL) in ASD. In a mutation screen of 158 multiplex families with ASD, Jamain et al (2003) identified the mutation, R451C, in the Neuroligin 3 gene (NLGN3) (Xq13) in two brothers from one family, and a nonsense mutation, D396X, in the Neuroligin 4 gene (NLGN4) (Xp22.3) in another family. A 2bp deletion in NLGN4 in a large French family with non-specific X-linked mental retardation has also been described. Previously, we demonstrated that the neuroligin mutations associated with ASD, NLGN3 R451C and NLGN4X D396X, result in loss-of-function and intracellular retention of the mutant proteins. Furthermore, over-expression of wild -type NLGN3 and NLGN4 in hippocampal neurons stimulates the formation of presynaptic terminals whereas the disease-associated mutations result in a loss of this synaptic function. Our findings suggest that mutations in neuroligin genes play an important role in neurodevelopmental defects. In this study, we searched for novel mutations in NLGN3 and NLGN4 by direct sequencing in addition to assaying for deletions in 99 ASD individuals from multiplex families obtained from the Autism genetic resource exchange (AGRE) consortium. All exons and exon-intron boundaries of NLGN3, NLGN4X and NLGN4Y were sequenced completely in 99 ASD subjects. To identify genomic rearrangements we have also analyzed microsatellite markers at Xp22, the region that contains the NLGN4X gene, and at Yq11.22, the region that contains the NLGN4Y gene. We report the frequency and functional consequences of mutations identified in NLGN3 and NLGN4 in 99 ASD subjects. This study was funded by the National Alliance for Autism Research.

Paraoxonase gene variants are associated with autism in Caucasian-American, and not in Italian families. *M. D'Amelio*¹, *I. Ricci*¹, *T.H. Wassink*², *L. Muscarella*³, *V. Guarnieri*³, *L. D'Agruma*³, *R. Militerni*⁴, *C. Bravaccio*⁴, *S. Trillo*⁵, *F. Montecchi*⁵, *M. Elia*⁶, *C. Schneider*⁷, *R. Melmed*⁷, *T. Pascucci*⁸, *S. Puglisi-Allegra*⁸, *K.L. Reichelt*⁹, *J. Piven*², *A.M. Persico*¹. 1) Mol Psychiatry & Neurogenetics, Univ Campus Bio-Medico, Rome, Italy; 2) Dept of Psychiatry, Univ. of Iowa Sch. of Med., Iowa City, IA and N.D.R.C., Univ. of North Carolina, Chapel Hill, NC; 3) Med. Genet. Serv., IRCCS Casa Sollievo dalla Sofferenza, S.Giovanni Rotondo (FG), Italy; 4) Child Neuropsychiatry, II Univ. of Naples, Italy; 5) Child Neuropsychiatry, IRCCS Osp. Bambino-Gesú, Rome, Italy;; 6) Neurology Serv., IRCCS Oasi Maria S.S., Troina (EN), Italy; 7) S.A.R.C., Phoenix, AZ; 8) Dept. of Psychology, Univ. La Sapienza, Rome, Italy; 9) Dept. of Pediatric Res., Rikshospitalet, Univ. of Oslo, Norway.

Our prior work points toward possible contributions of organophosphates (OPs) to autism pathogenesis in the U.S.A., and not in Italy. OP use in agriculture is equally intensive in both countries, whereas household use is much more intensive in the U.S.A. Within the framework of a gene-environment interactive model, genetically-vulnerable individuals prenatally exposed to OPs could undergo altered neuronal migration resulting in an autistic syndrome. Paraoxonase, the enzyme responsible for OP detoxification, is encoded by the PON1 gene located on ch 7q21.3. Functional SNPs located in the promoter and coding sequences of the PON1 (C-108T, L55M, Q192R) and PON2 (C311S) loci were genotyped in 163 Italian and 105 Caucasian-American trios. The R192 allele is associated with autism in the Caucasian-American and not in the Italian sample, using both case-control ($\chi^2 = 6.33$, 1df, $P < 0.05$) and TDT ($\chi^2 = 4.85$, 1df, $P < 0.05$) analyses. Specific haplotypes are associated with autism in Caucasian-American patients only ($T1^2 = 28.16$, $P < 0.01$). These data confirm our predictions, and support the hypothesis that the concurrence of genetic vulnerability and environmental exposure to OP compounds may yield autism in a sizable subgroup of North American patients. Supported by Telethon-Italy (GPP02019), the Fondation Jerome Lejeune, and the Cure Autism Now Foundation.

Program Nr: 2095 from the 2004 ASHG Annual Meeting

INVERSE REGULATION OF A7 AND A7-LIKE GENE EXPRESSION IN PREFRONTAL CORTEX: EVIDENCE OF ALTERATION IN SCHIZOPHRENIA. *V. V. De Luca, O. Likhodi, H. Van Tol, J.L. Kennedy, A.H.C. Wong.* CAMH, Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada.

The CHRNA7 gene is located on chromosome 15q13-14, a region linked with schizophrenia in several earlier studies. The 7-nicotinic receptor gene (CHRNA7) has a partial duplication of exons 5-10 including the intervening introns that map approximately 0.5 Mb proximal to the full-length CHRNA7 gene. On the 5' side of the duplicated exons are novel exons D-A, defining a hybrid gene (CHRFAM7A), which is transcribed, but it is not known whether it is translated. CHRFAM7A is transcribed in human brain and immortalized lymphocytes. The study was performed with postmortem brain obtained from the Stanley Foundation (Brodmann Area 46). CHRNA7 and CHRFAM7A mRNA levels were measured by quantitative real time RT-PCR to determine the expression relationship between CHRNA7 and CHRFAM7A in 35 schizophrenic, 35 bipolar and 35 healthy subjects. The mRNA levels of alpha7 and alpha7like genes seem to have an inverse relationship ($r=-0.36$; $p<0.01$). This negative correlation is weaker in schizophrenia ($r=-0.29$; $p=0.08$), suggesting an alteration in CHRNA7/CHRFAM7A equilibrium.

MUTATION SCREENING OF NRCAM AND IMMP2L, TWO CANDIDATE GENES FOR TOURETTES

SYNDROME AND OBSESSIVE COMPULSIVE DISORDER ON CHROMOSOME 7Q31. *R. Delorme¹, C.*

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Tourettes Syndrome (TS) is characterized by involuntary motor and vocal tics, whereas Obsessive-Compulsive Disorder (OCD) is characterized by recurrent, distressing unwanted thoughts and repetitive ritualistic behaviour. Both disorders have phenomenological and familial-genetic overlaps. Although no susceptibility genes for TS or OCD have been clearly identified, positive linkage and association studies have been reported. Chromosome 7q31 has been implicated in both disorders by the presence of a de novo duplication [46, XY,dup(7)(q22.1-q31.1)] in a proband with TS and the segregation of a translocation t(7; 18) (q31; q22.3) in a family with OCD and TS. Within 7q31, the inner mitochondrial membrane peptidase 2 like (IMMP2L) and the neural cell adhesion molecule (NrCAM) may represent suitable candidates for both disorders. Indeed, IMMP2L, disrupted in the duplication, has recently been shown to be associated with TS. NrCAM is an important mediator with multiple effects on the development of the neurons. Here, we report the mutation screening of the IMMP2L and NrCAM genes (promoter region and coding exons) in 24 individuals (16 diagnosed with TS and OCD and 8 with OCD and family history of TS). No variations were identified in the IMMP2L gene. In NrCAM, thirteen single nucleotide polymorphisms (SNPs) were identified: five in the intronic sequences and seven in coding regions. Of the exonic polymorphisms, only one changed the amino acid sequence (A526P). Linkage disequilibrium was calculated and the most informative SNPs were genotyped in 100 additional OCD patients and 95 controls. None of the SNPs showed association with OCD. In conclusion, this analysis strongly suggests that coding variations of IMMP2L and NrCAM do not play a major role in TS and OCD etiology. These results encourage us to screen additional candidate genes in the in the 7q31 region.

Program Nr: 2097 from the 2004 ASHG Annual Meeting

The effect of LDHA exon 5 genotype on mRNA production. *K. Dodelzon, S.L. Bradley, H.K. Sandhu, R.A. Philibert.*
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Panic Disorder (PD) is an anxiety syndrome that affects approximately 3% of the population and is characterized by recurrent discrete episodes of fear accompanied by psychological and/or physiological symptoms, most particularly respiratory distress. Previously, we have demonstrated a relationship between a haplotype encompassing exon 5 of Lactic Dehydrogenase A (LDHA), a gene that codes for a protein that serves as a convergence point for two means of inducing PD in individuals susceptible to developing the disorder, and ventilatory response to inhaled 5% CO₂. Intriguingly, while the sequence changes associated with the exon 5 haplotype variant do not affect primary amino acid sequence, they do change the sequence in a canonical exonic splice enhancer found in LDHA exon 5. To examine whether this exon 5 sequence change affects mRNA production, we used standard PCR and RT-PCR techniques to examine the mRNA production as a function of LDHA genotype in 150 independent lymphoblast cell lines and analyzed the resulting data using ANOVA analysis. Preliminary results from the first 60 of these samples suggest a trend for a relationship between exon 5 haplotype and LDHA mRNA production. In this presentation, we present the findings from the entire sample and discuss the possible relationship between LDHA exon 5 genotype and 1) altered respiratory response to increased levels of CO₂ and 2) vulnerability to Panic Disorder.

Genetic analysis of CYP1B1, MYOC, OPTN and OPTC genes in glaucoma patients from South India. S.K.

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Glaucoma represents a group of optic neuropathies with different genetic basis. In India, ~1.5 million people are blind due to glaucoma. Mutations in the MYOC gene at the GLC1A locus on chromosome 1q21-q31, CYP1B1 gene at the GLC3A locus on chromosome 2p21 and the OPTN gene at the GLC1E locus on chromosome 10p14 have been found in patients with glaucoma. The purpose of the present study was to screen sequence variations in these genes as well as in OPTC in a total of 146 glaucoma patients ascertained mostly from the state of Karnataka and its border areas with states of Andhra Pradesh and Tamilnadu, south India. Of these, 116 were affected with adult-onset primary open-angle glaucoma (A-POAG), 18 were with juvenile-onset primary open-angle glaucoma (J-POAG) and 12 were with primary congenital glaucoma (PCG). The coding regions of CYP1B1 and MYOC were screened for mutations and variations in all of the 146 patients using a combination of PCR-SSCP and DNA sequence analyses. Our analysis revealed a total of five mutations (two novel and three known) and seven variants/polymorphisms in CYP1B1. No mutation was found in MYOC. However, we have detected one known and one novel polymorphisms in this gene. Screening of 116 A-POAG patients showed one novel and one known risk-factor associated mutations in OPTN. In addition, two novel and one known polymorphisms were also detected in OPTN. Screening of OPTC in 116 A-POAG patients showed one novel and two previously known polymorphisms. (This work was supported by a grant from the CSIR, New Delhi).

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A protective role of HLA-A*02 in Multiple Sclerosis. *K. Duvefelt, B. Brynedal, E. Akesson, I. Roos, J. Hillert.*
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Multiple sclerosis (MS) is a chronic neurological disease affecting the central nervous system. The pathology is due to activated immune cells that are able to cross the blood brain barrier and attack myelin sheaths surrounding the axons. This leads to demyelination and disturbance of neurological functions. The cause of the disease is multifactorial and includes both genetic and environmental components. The HLA class II haplotype HLA-DRB1*1501,DRB5*0101,DQA1*0102,DQB1*0602 is the only genetic factor that consistently has been associated with MS. We have previously reported an association of the HLA-A gene with MS. The present study was conducted to further corroborate these data in a new and larger material within the same population. Genomic typing corresponding to serological specificities was conducted with allele-specific primers. HLA-A and DRB1 alleles were examined in 669 Swedish MS- patients and 715 controls (healthy blood donors). In this material we confirm that HLA-A*02 carriers have a decreased risk of developing MS (Odds ratio =0.47, two tailed P-value Fishers exact test= 7.2×10^{-12}). This association is independent of the well established HLA-DRB1*15 association (OR 3.4). Carrying both HLA-A*02 and HLA-DRB1*15 results in an OR of 1.15.

The wild-type form of the Tau protein is not degraded by the 26S proteasome. *S. Feuillette, O. Blard, M. Lecourtois, T. Frebourg, D. Campion, C. Dumanchin.* Inserm U614, Faculty of Medicine, Rouen, France.

Tau-positive inclusions in neurons are consistent neuropathological features of the most common causes of dementias like Alzheimer's disease and frontotemporal dementia. Ubiquitinated Tau positive inclusions have been reported in brain of Alzheimer's disease patients but the involvement of the ubiquitin-dependent proteasomal system in Tau degradation remains controversial. Therefore, we investigated whether the proteasome pathway is involved in endogenous Tau degradation both *in vitro* and *in vivo*. First, SH-SY5Y human neuroblastoma cells were treated with different proteasome inhibitors, ALLN, MG132, lactacystin and epoxomicin. Under these conditions, neither total nor phosphorylated endogenous Tau protein levels were increased. In contrast, an unexpected decrease of Tau protein was observed after treatment of cells with the proteasome inhibitors MG132, lactacystin and epoxomicin. The decrease of Tau that we observed was potentially related to apoptosis since we observed that this decrease simultaneously occurred to pro-caspase 3 and PARP cleavages. Second, we took advantage of a temperature sensitive mutant allele of the 20S proteasome in *Drosophila*. Genetic inactivation of the proteasome also resulted, in *Drosophila*, not in an increase but in a decrease of Tau. In conclusion, these results obtained *in vitro* and *in vivo* demonstrate that endogenous Tau is not degraded by the proteasome. Thus, it is likely that Tau, like the SOD1 protein becomes a substrate of the proteasome only in pathological conditions.

Association testing in patients with major depressive disorder and psychotic features. *B. Franke^{1,2}, R.J. Verkes², A. van Raaij¹, S. Hanssen¹, J. Janzing², J.K. Buitelaar², H. Scheffer¹.* 1) Dept Human Genetics, UMC Nijmegen, Nijmegen, Netherlands; 2) Dept Psychiatry, UMC Nijmegen, Nijmegen, Netherlands.

Major depressive disorder (MDD) is the leading cause of disability among those age five and older, and the second leading source of disease burden. MDD is mainly characterized by a depressed mood or a loss of interest and pleasure in daily activities for at least a two week period. Ten to 25% of women and 5 to 12% of men are affected by MDD within an entire lifetime. At any one point in time, 5 to 9% of women and 2 to 3% of men are likely to be clinically depressed. Psychotic features are observed in 10 to 15% of MDD patients. In addition to the symptoms of MDD, psychotic depression includes some features of psychosis, like hallucinations or delusions. The causes of MDD have not yet been identified but genetic factors are known to be involved. However, MDD represents a heterogeneous group of disorders and the genetic contribution to the disorder is likely to be very complex. Subclassification of patients or phenotype characteristics is therefore required before the genetics of MDD can be studied successfully. In the work presented here, we attempted to subclassify MDD by concentrating on MDD with psychotic features only. Eighty Dutch patients with well characterized MDD and psychotic features were compared with 150 healthy controls. Association testing was performed for frequent genetic variants in genes coding for transporters of the neurotransmitters serotonin (SLC6A4), norepinephrine (SLC6A2) and dopamine (SLC6A3). Genetic variants were chosen to represent the different haplotype blocks within the selected genes. At least two variants were analyzed for each of the genes and haplotypes were constructed. Genotype and allele frequencies were found to be similar to earlier studies involving these genetic variants. None of the variants or haplotypes tested appeared to be a major risk factor for MDD with psychotic features.

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Mutational screening of the ATP1A2 / FHM2 gene in ninety six individuals with sporadic or familial hemiplegic migraine (FHM), AHM, and EA phenotypes. *K. Gardner, C. Patankar, N. Pathe, W. Makhoul, M. Soso, S. Keryanov.*
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Background: Familial hemiplegic migraine has been associated with missense mutations in a Chr19p / FHM1 calcium channel gene CACNA1A and a Chr1q / FHM2 sodium potassium ATPase gene ATP1A2. Episodic ataxia (EA-2) has been associated with truncating mutations in the CACNA1A gene. Some families and individuals with episodic ataxia and alternating hemiplegia have clinical overlap with hemiplegic or other forms of migraine. Methods: Sporadic hemiplegic migraine individuals and small families (<2-3 FHM, AHM, EA) were evaluated after obtaining IRB approved consent using structured validated headache interview (KG), physical exam (KG) or record review (KG). Questionnaire data included demographic and past medical history, ethnic background, headache triggers, and medication response. Headache type was classified using I.H.S. criteria. Mutational analysis was performed in 4/ 23 ATP1A2 exons among sporadic individuals and selected family members after primer optimization, radiolabeled PCR amplification, electrophoresis on SSCP gels, and autoradiogram exposure. Conformers will be analyzed by PCR based sequencing. Results: Mostly sporadic hemiplegic migraineurs individuals were identified. Only one lacked a family hx of nonhemiplegic migraine or headache. Most referred with EA had additional migraine or family hx of migraine. Individuals from small families included 5 AHM individuals from 4 families, most with additional nonhemiplegic migraineurs. Two conformers were identified, one each from exons 16 and 19 in two with HM. The remainder of the 23 exons are under investigation with sequence variants to be described. Conclusion: Only a small number of genetic variants are identified in the previously reported mutant exons of the ATP1A2 gene among this enriched collection of sporadic or small family individuals with hemiplegic migraine, episodic ataxia, or alternating hemiplegia. This is consistent with expected complex inheritance for heterogenous disorders, especially in the setting of sporadic occurrence or small family size.

Genes Regulating the Adrenergic System and Childhood-Onset Mood Disorders. *L. Gomez¹, I. Burcescu¹, K. Wigg¹, N. King², A. Vetro³, E. Kiss³, K. Kapornai³, J.L. Kennedy², M. Kovacs⁴, C.L. Barr^{1,5}, The International Consortium for Childhood-Onset Mood Disorders.* 1) Toronto Western Research Institute, Toronto, Ontario, Canada; 2) Neurogenetics Section, Centre for Addiction and Mental Health, Toronto, Ontario, Canada; 3) Department for Child and Adolescent Psychiatry, Szeged University, Szeged, Hungary; 4) University of Pittsburgh, Pittsburgh, Pennsylvania, USA; 5) The Hospital for Sick Children, Toronto, Ontario, Canada.

The adrenergic system is implicated in the therapeutic mechanism of antidepressants and regulates a number of behavioural and cognitive functions that further implicate this system in the development of depression including attention, memory, response to stress, behavioral impulsivity and anxiety. Further, a recent genome scan of early-onset depression, identified the chromosomal locations of three of the adrenergic receptors; ADRA1B on 5q23-q33.3, ADRA2A, and ADRB1 on 10q24-q26, and ADRB3 on 8p12-p11.2, as linked to depression. In this study we examined the role of the adrenergic system in childhood-onset mood disorders (COMD) defined as age of onset < 14, using 195 nuclear families with 225 affected children collected in Hungary. We tested multiple polymorphisms across each of the genes for the adrenergic receptors; ADRA1A, ADRA2A, ADRA1B, ADRA1D, ADRA2B, ADRA2C, ADRB1, ADRB2, and ADRB3, for evidence of association using the transmission disequilibrium test. We found no significant evidence for association with the 23 markers genotyped in these genes thus far and therefore it is unlikely that the adrenergic receptors play a major role in the genetic susceptibility to COMD.

A polymorphism in the CYP46 gene affects the risk of Alzheimers disease in Finnish patients. *S. Helisalmi¹, S. Vepsäläinen¹, A.M. Koivisto^{1,2}, A. Mannermaa³, S. Iivonen¹, M. Hiltunen¹, H. Soininen^{1,2}.* 1) Neuroscience and Neurology, Kuopio University, Kuopio, Finland; 2) Department of Neurology, University Hospital, Kuopio, Finland; 3) Department of Pathology and Forensic Medicine, University Hospital and Kuopio University, Kuopio, Finland.

Background: CYP46 gene encodes cholesterol 24-hydroxylase, the rate-limiting enzyme catalyzing the conversion of cholesterol to 24S-hydroxycholesterol, which is the primary cholesterol elimination product in the brain. **Objectives:** To study if alleles of single polymorphic sites in the CYP46 gene have influence on the risk of the developing Alzheimers disease (AD). **Methods:** The frequencies of two single nucleotide polymorphisms (SNPs) and apolipoprotein E (APOE) were assessed in 422 AD patients and 469 cognitively healthy controls. Genotypes of the study groups were compared using binary logistic regression analysis. Linkage disequilibrium between markers was estimated and two-locus haplotype was constructed. **Results:** One SNP (rs754203) had significantly different allelic and genotypic frequencies between the AD and the control subjects. **Conclusion:** Taken together, these findings suggest that polymorphism(s) in the CYP46 gene increase risk for AD and support a potential role for CYP46 in AD.

Severe myoclonic epilepsy in infancy (SMEI) resulting from *SCN1A* and *GABRG2* mutations inherited from non SMEI parents. *S. Hirose*¹, *G. Fukuma*¹, *T. Kanaumi*¹, *S. Ueno*², *A. Ishii*¹, *Y. Haga*³, *A. Hamachi*¹, *M. Yonetani*¹, *M. Itho*⁴, *S. Takashima*⁵, *S. Kaneko*⁶, *A. Mitsudome*¹. 1) Dept Pediatr, Sch Med, Fukuoka Univ, Fukuoka; 2) Rehab Med, Inst Brain Sci, Sch Med, Hirosaki Univ, Hirosaki; 3) Dept Pediatr, Public Biyou Hosp Jimokujichyou; 4) Dept Mental Retard and Birth Defect Res, NCNP, Tokyo; 5) Yanagawa Inst for Dev Disabilities, Child Neurol, Interna Univ Health and Welfare, Yanagawa; 6) Dept Neuropsych, Sch Med, Hirosaki Univ, Hirosaki, Japan.

Mutations of genes encoding 1 subunit of a sodium channel (*SCN1A*) and 2 subunit of GABA_A receptor (*GABRG2*) have been reported to cause severe myoclonic epilepsy in infancy (SMEI). Most of such mutations were *de novo* in accordance with sporadic occurrence of SMEI, while febrile seizures and epilepsy are often seen in relatives of patients. Here we report two mutations of *SCN1A* and *GABRG2* which were found in individuals with SMEI and inherited from their parent who did not have SMEI. Both mutations were heterozygous and not found in 182 volunteers. One is a missense mutation of *SCN1A*, c.5045T>C:F1682S, which was identified in a girl with SMEI and her father with simple febrile seizures. F1682 was a highly conserved in Segment 5 in Domain IV of 1 subunit where is a hot spot for the mutations of SMEI. The other is a novel nonsense mutation of *GABRG2*, c.118C>T:Q40X, which was detected in dizygotic twin girls with SMEI and their apparently healthy father but not in their mother with seizures in childhood. The father's mutation was *de novo*. Electrophysiological studies of GABA_A receptors reconstituted with 1, 2 and 2 subunits on HEK cells revealed that mutant 2 with Q40X suppressed GABA-induced current values of the receptors. Immuno-staining brain specimens from one of the twins with anti GABA_A receptors indicated aggregations of the receptors in the somas of some neurons and neuropils. Our findings suggest that the full development of some of SMEI could also involve another putative genetic factor possibly inherited from the parents.

Linkage and association analysis of DNA variants in neuropeptide and neurotransmitter candidate genes in panic disorder. *L.M. Hodges¹, F. Haghighi², G.A. Heiman³, M. Durner³, O.V. Evgrafov³, C.T. Gilliam^{2,3}, S.E. Hodge³, J.A. Knowles^{2,3}, A.J. Fyer³, M.M. Weissman³, S.P. Hamilton¹.* 1) Dept of Psychiatry, University of California SF, San Francisco, CA; 2) Columbia Genome Center & 3) Dept of Psychiatry, Columbia University College of Physicians and Surgeons.

A clear genetic component underlies the complex etiology of panic disorder (PD) as evinced by family, segregation, and twin studies. The following neuropeptide and neurotransmitter pathway genes have all been implicated in animal studies of anxiety behavior or in targeted gene deletion experiments: gastrin-releasing peptide, gastrin-releasing peptide receptor, corticotropin-releasing hormone receptor, tachykinin receptor, and GABA receptor 5 and 3 subunits. In addition, pharmacological agents targeting a number of these gene products may be used to treat anxiety and PD. In support of these biological hypotheses we observe evidence for linkage in the genomic regions surrounding many of the candidate genes in recent unpublished genome-wide scan of PD. The high density of SNP markers derived from the Human Genome Project and characterized by the International HapMap Project allows the use of these markers for construction of informative haplotypes for linkage disequilibrium mapping studies of complex disorders. Using high throughput methods for genotyping SNPs (FP-TDI and fluorogenic 5-nuclease), we have employed a family-based design to investigate the role of these variants in the genetic susceptibility to PD. In 994 subjects, from 120 PD pedigrees, as well as 41 independent parent-proband triads, we genotyped an average of 5 SNPs per gene. Common and evenly distributed SNPs were chosen in order to cover the genomic regions of interest. Complementary statistical approaches of linkage and association analysis will be employed to test correlation between genetic variations in these genes and the susceptibility to PD. Both parametric (using recessive/dominant genetic models, three diagnostic models, and tests of heterogeneity) and nonparametric linkage analysis will be performed along with family-based association analysis. These data are currently being analyzed, and the results will be presented.

RARE VARIATIONS AND STOP MUTATIONS IN THE NEUROLIGIN GENES IN AUTISM AND

ASPERGER SYNDROME. *S. Jamain¹, H. Quach¹, C. Betancur², H. Goubran-Botros¹, C. Durand¹, J. Melke¹, R. Delorme¹, M. rastam³, C. Colineaux², IC. Gillberg³, H. Soderstrom³, B. Giros², D. Bonneau⁴, H. Firth⁵, M. Leboyer², C. Gillberg³, T. Bourgeron^{1 6}.* 1) neuroscience, Institut Pasteur, Paris, IDF, France; 2) Inserm U513, Creteil, France; 3) Goteborg University, Goteborg, France; 4) CHU Angers, Angers, France; 5) Addenbrooke's Hospital, Cambridge, UK; 6) Univeristy Paris 7, Paris, France.

Autism is characterised by qualitative impairments in social interactions and communication and associated with restrictive interests and repetitive behaviours. In a recent paper, we reported mutations in X linked neuroligins (NLGN3 and NLGN4) in subjects with autism and Asperger syndrome. The association of neuroligins with autism was recently also supported by the identification of a frame-shift mutation of the NLGN4 gene in one large family with mental retardation and autism. Neuroligins are cell adhesion molecules that contribute to the formation of synapses In humans, five neuroligins have been described and all of them are localised in chromosomal regions previously associated with autism. Here, we report the analysis of the five NLGN genes in individuals with autism. To investigate whether these genes may have a role in autistic disorder, we systematically screened all neuroligin genes for mutations in the coding sequence in 90 (NLGN1, NLGN2) to 290 (NLGN3, 4 and 4Y) individuals affected with autism or Asperger syndrome. In addition, single nucleotide polymorphisms (SNPs) identified in this study were analysed for association with autism.

Defining a locus for holoprosencephaly on human chromosome 14 and characterization of candidate genes. *D.*

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Twelve Holoprosencephaly (HPE) loci have been proposed on 11 chromosomes, with four genes mapping to these loci found mutant among patients. We have defined a locus for holoprosencephaly on human chromosome 14q13, denoted as HPE8, between the markers D14S49 and AFM205XG5 by mapping the deletion intervals of 6 affected patients. Annotation of 3.5 Mb genomic sequence within this interval identified 23 possible genes. We identified five possible candidate HPE genes, namely, NPAS3, C14ORF10, C14ORF11, C14ORF19 and SNX6 that are expressed in human fetal brain (20 to 33 wks gestation). The cDNA sequences encoding the full or partial open reading frames were isolated by combinations of RACE and cDNA library screenings on human fetal brain cDNA libraries. The NPAS3 gene was excluded for further analyses since this gene was found disrupted in a family affected with schizophrenia. The C14ORF10 and C14ORF19 genes were also tentatively excluded for further analyses since a partial human cDNA sequence was obtained and/or the function was characterized in a model organism that is inconsistent with our current criteria for a candidate gene for holoprosencephaly. The murine orthologs of the remaining two HPE candidates (C14ORF11, SNX6) were shown expressed using RT-PCR, as early as 7 dpc to 12 dpc in whole embryos, with continued expression in the head from 14 to 18 dpc. Whole mount in situ hybridization on 11 dpc murine embryos showed both *Snx6* and *C14orf11* genes were expressed in the developing central nervous system and other extra-cranial tissues. Northern analysis on human adult multi-tissue blots showed ubiquitous expression in several adult and brain tissues of a 1.7 kb transcript for C14ORF11, and 3.4 kb transcript for SNX6. Cellular localization studies with C-EGFP fused HPE proteins demonstrated the SNX6 and C14ORF11 proteins were localized in the cytoplasm of COS1 and transformed skin fibroblasts.

BDNF variants are associated with Parkinson's disease: the GenePD study. *S. Karamohamed¹, J. Latourelle¹, J.B. Wilk¹, A.L. DeStefano¹, B. Racette², J.S. Permuter², R.H. Myers¹, A. Parsian³.* 1) Dept Neurology, Boston Univ Sch Medicine, Boston, MA; 2) Department of Neurology, Washington University School of Medicine, MO; 3) Human Genomics Laboratory, University of Arkansas for Medical Sciences, AR.

Parkinson disease (PD) is the second most common progressive neurodegenerative disorder, with a prevalence of approximately 1% at the age of 65, increasing to 45% by the age of 85. The role of genetics in early-onset PD has been established, but the genetic contribution for the more common late-life form remains less clear. One prominent candidate gene is the brain-derived neurotrophic factor (BDNF) on chromosome 11p13. BDNF is a trophic factor for dopaminergic neurons and protects nigral dopamine neurons in animal models of PD. We investigated five single nucleotide polymorphisms (SNPs) in the BDNF gene in 611 familial PD cases (581 with known onset ages) from 329 families from the GenePD study and 249 controls. We typed one SNP in the promoter region (C-1331T), two intronic SNPs (rs2049045, Celera SNP C1751792), and two coding SNPs (C270T and G198A). Three SNPs (rs2049045, C1751792, and G198A) spanning 14 Kilo base region, were associated with PD onset age. The G198A SNP codes for a nonsynonymous amino acid substitution (M66V). Homozygosity for the rare allele of G198A (A) is associated with a 5.4 year later onset ($P = 0.0001$). Homozygosity for the rare allele of C1751792 (GG) was associated with 4.6 years later onset ($P = 0.007$), and homozygosity for the rare allele of rs2049045 (C) with a 5.2 year later onset ($P = 0.005$). A haplotype of these three did not show stronger association than the individual SNPs. Conversely, while no individual SNP showed association to PD affection, the haplotype of C-1331T with rs2049045; and C1751792 or G198A was associated with affection ($P < 0.05$). Studies to explore the effects of these SNPs on gene expression and sequencing for additional SNPs is in progress. The findings support the hypothesis that BDNF variants or expression plays an important role in influencing PD onset or risk.

Mutations in BBS proteins cause anosmia in humans and defects in olfactory cilia structure and function in the mouse. *N. Katsanis¹, H.M. Kulaga¹, C.C. Leitch¹, E.R. Eichers², J.L. Badano¹, A. Lesemann¹, J. Hill³, B.E. Hoskins³, J.R. Lupski², P.L. Beales³, R.R. Reed¹.* 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Baylor College of Medicine; 3) University College London.

Defects in cilia have been associated with several human disorders including Kartagener syndrome, polycystic kidney disease, nephronophthisis and hydrocephalus. We proposed recently that the pleiotropic phenotype of Bardet-Biedl syndrome (BBS), which encompasses retinal degeneration, truncal obesity, renal and limb malformations and developmental delay is due to dysfunction of basal bodies and cilia. Here we show that BBS patients manifest partial or complete anosmia. To test whether this phenotype is due to ciliary defects of olfactory sensory neurons, we examined the olfactory and respiratory epithelia of mice ablated for each of *Bbs1* or *Bbs4*. Loss of function of either BBS protein results in a dramatic phenotype of the olfactory, but not the respiratory epithelium, including severe reduction of the ciliated border, trapping of olfactory ciliary proteins in dendrites and cell bodies, downregulation of expression of olfactory markers and disorganization of the dendritic microtubule network that coincides with the mislocalization of the BBS-interacting protein PCM1. Our data provide the first *in vivo* evidence of the role of BBS proteins in the microtubule organization of mammalian ciliated cells and indicate that anosmia might be a useful determinant of other pleiotropic disorders with a suspected ciliary involvement.

Genome-wide microsatellite association studies for Alzheimers disease. *H. Kawakami¹, H. Morino¹, H. Terasawa¹, M. Oda¹, H. Maruyama¹, T. Miyachi¹, K. Sasaki², Y. Izumi³, R. Kaji³, G. Tamiya⁴, H. Inoko⁴, M. Matsumoto¹.* 1) Clinical Neuroscience and Therapeutics, Hiroshima Univ Graduate Sch Biomedical Sci, Hiroshima, Japan; 2) Kinoko Espoir Hospital, Kasaoak, Okayama; 3) Clinical Neuroscience, Tokushima University, Tokushima; 4) Molecular Life Science, Sch Medicine, Tokai University, Isehara, Kanagawa.

The most common form of dementia in the elderly is Alzheimer disease (AD) that is believed to be genetically complex. It has been established that apolipoprotein E contributes to late-onset AD. However, other genetic factors contributing to AD are still unknown. Therefore, we investigated the susceptibility gene of AD. We performed the genome-wide association study using about 24,000 of microsatellite markers as a tool for identifying disease susceptibility regions. One hundred and sixty AD patients were pooled for the 1st set. Similarly, 160 healthy individuals were pooled for the 1st set as controls. We performed Fishers exact test and a significant difference was determined when a p-value was less than 5%. About 80 % of microsatellite markers obtained the data. After the analysis, 11.6 % of markers showed significant differences. We will perform the further screening of the 2nd set and the 3rd set pooled samples using these positive markers.

Opposite effects of genetic background on the acoustic startle response in the fragile X knockout mouse. R.F.

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Fragile X knockout mice, a validated animal model for the human mental retardation disorder were subjected to the Acoustic Startle Response (ASR). This brainstem reflex may serve as a neuronal basis for behavior. The magnitude of this sudden response to a loud noise was measured. At different sound levels of 90, 100 and 110 dB, fragile X knockout mice in an inbred C57BL/6 knockout background showed a 48% increase, no significant difference, and a 16% decrease in ASR magnitude when compared to wild type littermates, respectively. In C57BL/6 x 129P2/OlaHsd F1 hybrids at sound levels of 100 and 110 db, a 32% and 14% decrease in ASR of the knockout mice when compared to wild type littermates was measured, respectively. In contrast, F2 intercross knockout mice showed a 13% and a 10% increase in ASR, respectively. The opposite effect caused by the difference in genetic background is remarkable as, to our knowledge, hardly any test has been described in which opposite effects of a knockout mutation are caused by differences in genetic background. This case is especially remarkable, as the F1 and the F2 generation both consist for 50% of C57BL/6 and for 50% of 129P2/OlaHsd. Only the distribution along the chromosomes differs: the F1 generation has one chromosome of each parental strain, whereas in the F2 generation the distribution of the genomic material is semi-randomly. We believe that such differences may be the consequence of modifier genes in either the C57BL/6 or the 129P2/OlaHsd background, and attempt to map these using a large population of F2 knockout hybrids using the wild type littermates as controls.

Investigation of Dopamine D1 Receptor Pathway-Associated Genes in Attention Deficit/Hyperactivity Disorder.

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Attention-deficit hyperactivity disorder (ADHD) is characterized by marked inattention, hyperactivity, and impulsivity. A dopamine system dysfunction has been proposed as an underlying factor of the disorder. Indeed, positive association and/or linkage have been found for genes in the dopamine pathways, including the dopamine receptor D1 and D5 (DRD1 and DRD5). In this study, we have tested two genes involved in the DRD1/5 signaling pathway as candidates for ADHD susceptibility: PPP1R1B, which encodes DARPP32, a major target for dopamine-activated adenylyl cyclase and the CALCYON gene which encodes a brain-specific DRD1/5-interacting protein. Three bi-allelic polymorphisms in PPP1R1B and six in the CALCYON gene were genotyped in 215 nuclear families. Association was assessed with the transmission/disequilibrium test, using ETDT for individual alleles and TRANSMIT for haplotypes. Quantitative trait analyses were performed using FBAT in relation to ADHD symptom scores. No association was found between the three DARPP32 polymorphisms and ADHD and no biased transmission was observed for any of the DARPP32 haplotypes. CALCYON analysis, however, has shown significant evidence for biased transmission of two polymorphisms, found in the 5' and intronic regions of the gene ($\chi^2=5.042$, 1df p=0.025 and $\chi^2=4.738$, 1df p=0.030). Quantitative traits analyses demonstrated that these polymorphisms are associated with both the inattentive and hyperactive/impulsive symptoms of ADHD. In addition, two CALCYON haplotypes demonstrated significant evidence for biased transmission ($\chi^2=4.79$, 1df p=0.029 and $\chi^2=4.67$, 1df p=0.030) and significant relationships were also found with both dimensions of the disorder. In summary, these findings do not support DARPP32 as a susceptibility factor in ADHD. However, our data suggest that the CALCYON gene may be involved in ADHD and needs further studies.

Variations in the brain-derived neurotrophic factor (BDNF) gene and susceptibility to bipolar I disorder. *F.W. Lohoff¹, T. Sander², J. Gallinat², W.H. Berrettini¹*. 1) Psychiatry, University of Pennsylvania, Philadelphia, PA; 2) Max Delbrück Centrum, Gene Mapping Center, Berlin, Germany.

Background: Recent studies have indicated that the brain-derived neurotrophic factor (BDNF) gene is involved in the etiology of bipolar disorder (BPD). The BDNF gene maps to the short arm of chromosome 11, a region where linkage studies have suggested a putative locus for BPD. Two family based association studies showed that the functional polymorphism Val66Met in the BDNF gene is associated with BPD, however others could not confirm results. Here we performed a replication study in an independent sample and tested the hypothesis that the Val66Met variation and four additional variations in the BDNF gene confer susceptibility to BPD.

Methods: 627 bipolar I patients and 998 healthy controls were genotyped for the Val66Met polymorphism. Four additional SNPs across the BDNF gene were genotyped in 345 patients and 266 controls, using Applied Biosystems assay-on-demand SNP genotyping assays as per manufacturers protocol. Genotypes and allele frequencies were compared between groups using Chi square contingency analysis. LD was calculated and haplotypes were estimated using the cocophase program.

Results: Allele frequencies of the Val66Met polymorphism differed significantly between BPD patients and controls ($\chi^2=4.4$; $df=1$; $p=0.036$; $OR=1.21$). All genotype counts were in Hardy-Weinberg equilibrium. We observed strong LD in the 3' end of the gene but could not demonstrate LD in the 5' end of the gene. Haplotype analysis showed that combination of rare alleles in the 5' end of the gene differed significantly between patients and controls ($p=0.01$; $OR=0.54$).

Conclusions: These results suggest that the Val66Met polymorphism in the BDNF gene might increase susceptibility to BPD in the Caucasian population. However, rare haplotypes in the 5' end of the gene which are not in LD with the Val66Met SNP, might decrease susceptibility to BPD. Further studies are necessary to elucidate the involvement and pathophysiology of BDNF in BPD.

Metallothionein genes (MT-I, -II and -III) and ethanol preference: studies involving expression in the brain and MT-I/MT-II knockout mice. *K.D. Loney, R.K. Uddin, S.M. Singh.* Molecular Genetics Unit, Department of Biology and Division of Medical Genetics, University of Western Ontario, London, Ontario, Canada N6A 5B7.

Variability in ethanol preference phenotypes of mouse strains offers a suitable model for studies on this endophenotype of alcoholism and fetal alcohol syndrome. We have hypothesized that it may be determined by gene expression profiles in the brain (Treadwell, Pagniello and Singh, 2004). Further, we have reported (Loney, Uddin, and Singh, 2003) that metallothionein-II (MT-II) is an ethanol-responsive gene in the brain, with basal mRNA levels positively correlating with the ethanol preference phenotypes of four inbred mouse strains (A/J, BALB/cJ, DBA/2J; alcohol avoiders, and C57BL/6J; alcohol preferring). In this study we report novel results on MT-I and -III gene expression in the brain and explore the involvement of MT-II in ethanol preference determination through MT-I/MT-II gene knockouts. Results of semi-quantitative RT-PCR have shown MT-I to be an ethanol-responsive gene in all four strains of mice (1.3-1.9X), with C57BL/6J showing the smallest increase in mRNA level following ethanol treatment. The brain-specific MT-III was found to be unresponsive to ethanol, but interestingly C57BL/6J, the alcohol preferring strain, appeared to have the lowest mRNA levels when compared the three alcohol avoiding strains, which were observed to have 1.3-1.5X greater levels. In addition to expression studies, results from two-bottle choice tests with 10% ethanol and water have revealed that MT-I/MT-II knockouts in the 129/Sv background have a slightly higher measure of ethanol preference compared to their wild-type controls. This finding, while interesting, is contrary to our hypothesis that decreased MT-II expression would result in a decrease in ethanol preference. Taken together, these results continue to offer the possibility that the metallothionein gene family may be involved in alcohol-related phenotypes, such as ethanol preference. However, this association is not direct and may depend on the interacting genes and background genotypes. Supported by CIHR, NSERC, and Ontario Mental Health Foundation.

Genetic characterization of human Citron in bipolar disorder. A.M. Lyons-Warren¹, J. Chang¹, M. Garant³, M.G. McInnis¹, A. Sawa^{1,2}. 1) Psychiatry, Johns Hopkins University, Baltimore, MD; 2) Neuroscience, Johns Hopkins University, Baltimore, MD; 3) Division of Endocrinology, Diabetes & Nutrition, University of Maryland Baltimore.

DISC-1 (Disrupted in Schizophrenia-1) has recently been associated with bipolar disorder (BP) (Maeda et al, this meeting 2004). Yeast two-hybrid analysis of DISC-1 revealed several protein interactors, possibly as part of a signaling pathway. Citron was selected from among these DISC-1 interactors for further investigation because the gene coding for *Citron* is located on chromosome 12q24, a reported locus for BP in several linkage studies. Citron is a cytoskeletal synaptic protein that plays a key role in cytokinesis, neurogenesis, and glutamatergic neurotransmission. The sequence for human *Citron* was not available; therefore, we compiled it from contig sequences available from NCBI and UCSC databases. The human gene contains 6,105 base pairs divided into 47 exons, as well as 6 protein domains including a kinase domain and a DISC-1 binding domain. Human and mouse *Citron* exhibit a 96% homology. We next conducted a family-based association study of *Citron* and BP. Select regions, chosen based on functional importance, were directly sequenced in BP patients from families with evidence of linkage to 12q24. This analysis resulted in the discovery of a novel single nucleotide polymorphism (SNP) in exon 23 of human *Citron* gene and confirmed the existence of a second SNP, also in exon 23. We then extended the study to span the entire gene, including 7 additional SNPs from the NCBI database. Our initial association study of these 9 SNPs in samples from 65 BP pedigrees revealed a marginally significant association (P0.02) with BP for one haplotype. Thus, we have characterized and provided the initial study of human *Citron*.

Multifactor-Dimensionality Reduction (MDR) reveals gene-gene effects on GABA_A receptor subunit genes. *D.Q. Ma¹, H. Mei², A.E. Ashley-Koch¹, E.R. Martin¹, P.L. Whitehead¹, M.M. Menold¹, M.D. Ritchie³, G.R. DeLong⁴, R.K. Abramson⁴, H.H. Wright⁴, M.L. Cuccaro¹, J.P. Hussman⁵, J.R. Gilbert¹, M.A. Pericak-Vance¹.* 1) Duke university medical center, center for human genetics, Durham, NC; 2) North Carolina State University, Raleigh, NC; 3) Vanderbilt University, Nashville, TN; 4) University of South Carolina, School of Medicine, Columbia, SC; 5) Hussman Funds, Ellicott City, MD.

Autism is complex genetic disorder likely due to a combination of genetic and environmental factors and their associated interactions. GABA is the primary inhibitory neurotransmitter in adult brain. Eight GABA classes (α1, α2, α3, α4, α5, α6, α7, and α8) and twenty receptor subunit genes have been characterized in mammals. Multiple lines of evidence implicate GABA_A receptor (GABAR) systems in autism. The GABA Autism Project will test all known GABAR subunits and selected GABAergic related genes to identify genetic factors associated with autism risk: RG1| LOC16, GABRA2, GABRA4 and GABRB1 on 4p12, GABRB2, GABRA6, GABRA1, GABRG2 and GABRP on 5q34-q35.1, GABRR1 and GABRR2 on 6q15, as well as GABRA5, GABRB3 and GABRG3 on 15q12. Intronic and silent mutation SNPs within each gene were analyzed in 399 Caucasian autism families. MDR was employed to identify the best model among all possible locus combinations in each chromosome. Prediction error and consistency for each model were considered to identify the potential locus for further test of the interaction among genes cross chromosomes. MDR revealed statistically significant 3-locus effect on all 4 chromosomes (chromosome4:rs1497571,rs2280073,rs6289 within RG1|LOC16,GABRA2 and GABRB1,p=0.011;chromosome5:rs2962407,hcv11814555, hcv3165046 within GABRB2,GABRA1 and GABRP gene, p 0.000; chromosome6: rs423463,hcv9866022,rs2148174 within GABRR1 and GABRR2 gene,p=0.001;chromosome15: rs1426217, hcv42974,hcv2078506 within GABRB3,GABRA5 and GABRG3 gene,p=0.031). Moreover,MDR showed a significant multi-locus effect with hcv11814555 (GABRA1) on chromosome5 and hcv42974 (GABRG3) on chromosome15(p=0.001). These findings suggest that GABAergic genes are significant contributors to autism risk and may involve complex interactions.

NMDA receptor NR2B subunit gene GRIN2B in schizophrenia and bipolar disorder: polymorphisms and expression levels. *L. Martucci, A.H.C. Wong, V. De Luca, O. Likhodi, G.W.H. Wong, N. King, J.L. Kennedy.* Dept Neurogenetics, CAMH, Univ Toronto, Toronto, ON, Canada.

The NR2B protein subunit appears to be critical for a number of the basic structural and functional attributes associated with the NMDA receptor. We studied three polymorphisms located in the gene for NR2B, namely GRIN2B: G-200T, located in the 5'UTR, and the A5806C and T5988C, located in the 3'UTR. We tested the association between these markers and schizophrenia using a sample consisting of 192 case control pairs matched by age, gender, and ethnical background, and a sample of 86 trios. The same markers were studied in our sample of 318 bipolar disorder trios, of which 158 probands displayed psychotic symptoms. We also studied post-mortem cortical mRNA levels of GRIN2B in groups of 35 each of SCZ, BP and healthy controls; DNAs from these groups were also genotyped for the G-200T marker. Our schizophrenia case-control sample and the SCZ triad sample combined show association of the G allele of this marker with the disease ($p=0.0034$). The T5988C marker was associated with BP disorder ($p=0.02$), whereas the A5806C marker showed positive association with those among the bipolar patients who had psychotic symptoms ($p=0.0038$). The haplotype analyses also showed preferential transmission of haplotype G-C-T in BP disorder and of haplotype T-C-C in SCZ. The analysis of overall haplotype frequencies in the case control sample showed marked differences between cases and controls ($p=0.01$). We found no difference in GRIN2B expression levels between schizophrenia, bipolar disorder and healthy controls. No association was present between those groups and either allele of the promoter marker. The gene expression sample, however, is significantly smaller compared to our Toronto based samples and thus might have insufficient power to detect subtle changes both in expression levels and genotype. Other confounding factors such as chronic disease state and antipsychotic treatment could have altered these findings. These results overall support the hypothesis of a role of GRIN2B in the pathogenesis of schizophrenia and of bipolar disorder.

Expression profiles for 6p reading disability locus (DYX2) gene-candidates. *H. Meng¹, S.D. Smith², J. Kenyon², K. Hager³, M. Held¹, J.C. DeFries⁴, R.K. Olson⁴, B.F. Pennington⁵, J. Gelernter⁶, G.P. Page⁷, J.R. Gruen¹.* 1) Dept Pediatrics, Yale Child Health Research Center, Yale Univ, New Haven; 2) Munroe Meyer Institute, Univ of Nebraska Medical Center, Omaha; 3) WM Keck Foundation Biotechnology Resource Laboratory, Yale Univ, New Haven; 4) University of Colorado, Boulder; 5) University of Denver, Denver; 6) Department of Psychiatry, Yale Univ, New Haven; 7) Department of Biostatistics, University of Alabama at Birmingham, Birmingham.

Reading disability (RD), or dyslexia, is the most common neurobehavioral disorder affecting children, with a prevalence rate of 5 to 17.5%. An expanding large body of evidence indicates that RD is genetically based, with heritability between 0.4 and 0.6. We recently described a peak of association at marker JA04 (Kaplan et al, 2002, *AJHG*, 70: 1287) with orthographic choice ($\chi^2_{sq} = 11.49$, $p = .0007$) on 6p21.3-22, where several groups have independently found linkage. We also identified several candidate genes in the vicinity of JA04 that are strongly expressed in human brain (Londin et al, 2003, *BMC Genomics*, 4: 25). To distinguish which candidate gene could be responsible for the peak of association, and would warrant further interrogation for alleles that could render susceptibility to RD, we developed a panel of 147 single nucleotide polymorphism (SNPs) spanning a 1.2Mb region around JA04. Markers were chosen from the public domain (dbSNP) and Celera databases. We genotyped 154 RD families ascertained at the Colorado Learning Disabilities Research Center (Boulder) by TaqMan and pyrosequencing. Markers were tested for both transmission disequilibrium and total association with 11 reading performance phenotypes. These data localize the association peak to a narrow region spanning 500kb and 6 genes with poor performance in several reading tests. We also assessed expression of each gene in 18 sub-regions of human brain by real time RT-PCR. The location of the association peak and expression patterns implicates 3 genes in particular that warrant further interrogation for RD allele(s).

Increased BDNF plasma levels in patients with eating disorders are associated to the -270C/T polymorphism.

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Anorexia nervosa (AN) and bulimia nervosa (BN) are complex psychiatric conditions where genetic and environmental factors are involved. Murine models and association studies in patients with eating disorders (ED) suggest a potential role of brain-derived neurotrophic factor (BDNF) in eating behavior. We have reported a consistent association of BDNF single nucleotide polymorphisms (SNPs) and ED. The 270C/T BDNF SNP is associated to BN, and the Val66Met variant to both AN and BN in six different European populations. We present here the analysis of BDNF levels in blood of patients with AN and BN that have been genotyped for BDNF SNPs. We assessed the BDNF blood concentration in a clinical sample of 118 Spanish Caucasoid ED patients (54 AN and 64 BN) and 68 sex-matched unrelated controls. BDNF levels were significantly higher in both AN (46.725.9 ng/ml) and BN (41.023.4 ng/ml) patients than in control subjects (16.45.7 ng/ml; $p < 0.0005$). We did not find a significant effect of the Met66 functional variant on BDNF levels, as both Met66 carriers and non-carriers showed increased BDNF concentrations. In contrast, mean BDNF levels were significantly higher in ED patients homozygous for the 270C variant (4924.2; $p = 0.012$) than those carrying the 270T allele (26.917.245), which showed BDNF levels similar to controls. The altered BDNF plasma levels observed in this study provide physiological evidence of a possible role of this neurotrophic factor in AN and BN, and strongly argues for its involvement in eating behavior and body weight regulation.

Large scale association analysis for identification of genes underlying late-onset Alzheimers disease. *T. Miki¹, H. Yamagata², K. Taguchi¹, W. Zhong¹, K. Kamino³, Y. Akatsu⁴, Y. Tabara², I. Kondo².* 1) Dept of Geriatric Medicine, Ehime Univ Sch of Medicine, Onsegun, Ehime, Japan; 2) Dept of Medical Genetics, Ehime Univ Sch of Medicine, Onsegun, Ehime, Japan; 3) Division of Psychiatry and Behavioral Proteomics, Osaka Univ, Suita, Japan; 4) Choju Medical Insitute, Fukushima Hospital, Toyohashi, Japan.

Alzheimers disease (AD) is a complex disease with both environmental and genetic determinants. To date, most studies of AD genetics have focused on a candidate gene approach in unrelated cases and controls, a design that is more powerful than linkage analysis in families when examining a specific gene of interest. Many vascular risk factors such as hypertension, diabetes mellitus, and hypercholesterolemia have recently been shown also to increase the risk of AD. To investigate large scale association analysis for identification of AD genes, we chose a total of 230 candidate genes (67 reported AD-related genes, 163 atherosclerosis-related genes) and carried out high throughput genotyping (TaqMan-probe method) for SNPs in 380 late-onset AD and 380 controls. A total of 3/67 (4.5%) atherosclerosis-related genes and 10/163 (6.1%) AD-related genes showed significant association (p 0.05). Most AD-related genes including IL10, GSK3B, APP, PEN2, PS1, PS2, A2M, HMMR, ACE, BACE, SNCA showed no association. Positive chromosomal loci were 1, 2, 3, 7, 8, 9, 10, 11, 12, 13, 15, 17, 19, 21. Our results indicate some of AD-risk genes may include atherosclerosis-related genes.

SCA8 BAC transgenic mice: cerebellar dysfunction and 1C2/ubiquitin positive neuronal inclusions. *M. Moseley*¹, *Y. Ikeda*¹, *W. Gao*², *M. Weatherspoon*¹, *M.T. Su*³, *H.M. Hsieh-Li*³, *G.J. Lee-Chen*³, *G. Chen*², *K.C. Reinert*², *T.J. Ebner*², *J.W. Day*¹, *L.P.W. Ranum*¹. 1) Inst Human Genetics; 2) Dept Neuroscience, Univ MN, Minneapolis, MN; 3) Dept Life Science, Natl. Taiwan Nml. Univ, Taipei, Taiwan.

Spinocerebellar ataxia type 8 (SCA8), a slowly progressive ataxia, is caused by a CTG expansion. To understand the molecular mechanism of SCA8 we developed a transgenic mouse model using a human BAC clone with the normal (11 CTGs) or expanded (118 CTGs) SCA8 repeat tract. Two high copy expansion lines have a progressive, severe phenotype of impaired locomotion, weight loss and death (14 to > 52 wks). A more slowly progressive, later onset (~12-18 mos) phenotype affecting gait is observed in all 5 remaining expansion lines with no similar phenotype in 3 control transgenic lines. No evidence of cerebellar or CNS degeneration was found in affected mice, although *in vivo* optical imaging studies demonstrate neuronal dysfunction of the parallel fiber-Purkinje cell circuit (with reduced GABA-ergic inhibition) in both high and low copy expansion but not control lines. 1C2 and ubiquitin positive nuclear inclusions were found in Purkinje and pontine neurons of the high copy number expansion but not control transgenic animals. These results parallel our observation of 1C2 inclusions in human autopsy tissue from our large SCA8 family (see Ikeda *et al.*). The 1C2 antibody has been reported to recognize both glutamine and leucine expansions. The known SCA8 transcripts have a short ORF encompassing the CTG repeat that conceptually translates a poly-leucine tract. Although we have not detected a transcript in the CAG direction, a polyQ expansion remains a formal possibility. Alternatively, CUG containing SCA8 transcripts may be noncoding and act through an RNA mechanism similar to FXTAS in which CGG containing transcripts induce the formation of 1C2 negative but ubiquitin positive inclusions, however we have not detected CUG RNA foci in SCA8 brains. Our results demonstrate that the SCA8 CTG repeat expansion causes a neurological phenotype affecting the physiology of the cerebellum in mice and this phenotype relates to that of the human disease by the presence of 1C2-positive, intranuclear inclusions.

The BDNF gene is associated with rapid cycling bipolar disorder. *D.J. Mueller¹, V. De Luca¹, T. Sicard¹, N. King¹, M. Lanktree¹, J. Strauss¹, E. Mundo², F. Macciardi¹, P. Muglia¹, J.L. Kennedy¹.* 1) Neurogenetics Section, Centre for Addiction and Mental Health (CAMH), University of Toronto, Toronto, ON, Canada; 2) Department of Psychiatry, University of Milan, Italy.

We have previously reported significant associations between the Val66Met and GT(n) repeat polymorphisms of the BDNF gene with bipolar disorder (BD). However, these findings have not consistently been replicated which may be explained by considerable methodological heterogeneities among studies. In our sample of 312 nuclear families (with at least one offspring affected with BD) we have added four novel SNP markers (rs3763965, hCV11592756, rs2049045, rs2140887) located within or in the vicinity of the BDNF gene and performed TDT and haplotype analyses. Furthermore, we investigated whether our findings may be determined by the phenomenon of rapid cycling (RC) that has been present in 58 patients (19%). RC is defined by the occurrence of four or more (depressive and/or manic) mood episodes within 12 months. Our analyses revealed that both the hCV11592756 and the rs2049045 SNPs were significantly associated with BD using TDT analyses ($p=0.02$ and $p=0.009$ respectively). These two SNPs appear to belong to a haplotype block flanking the Val66Met and GT(n) repeat polymorphisms. The haplotype including the hCV11592756 (A-allele), Val66Met (G-allele), GT(n) repeat (allele 3) and rs2049045 (G-allele) proved to be over transmitted (49:25) in patients with BD ($p=0.008$). Interestingly, and despite the reduced sample size and reduced power, TDT analyses revealed significant associations with similar or higher likelihood ratio scales with all four SNPs in the sample of patients with RC as compared to the total sample. Conversely, removing patients with RC from the total sample led to non-significant findings with all four SNPs. Our findings suggest that the BDNF gene is likely to be associated with a specific homogeneous subgroup of patients with BD, namely RC. Thus, non replications in studies focusing on the BDNF gene and BD may either be due to a non-distinction of RC and/or limited samples and power. Our observations should be taken into account in studies dealing with the BDNF gene in mood disorders.

The social deficits of the secretin receptor knockout mouse: animal models relevant to autism. *I. Nishijima¹, T. Yamagata², C. Spencer³, O. Alekseyenko³, E. Weeber⁴, J. Sweatt⁴, M. Momoi², R. Paylor³, D. Armstrong⁵, D. Nelson³, A. Bradley⁶.* 1) Department of Pediatrics, The Ohio State University, Columbus, OH; 2) Department of Pediatrics, Jichi Medical School, Tochigi, Japan; 3) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 4) Division of Neuroscience, Baylor College of Medicine, Houston, TX; 5) Department of Pathology, Baylor College of Medicine, Houston, TX; 6) The Wellcome Trust Sanger Institute, Cambridge, UK.

Autism is a neurodevelopmental disorder marked by behavioral features and linguistic development problems. Secretin is a peptide hormone that is secreted from the duodenum to stimulate the pancreas to secrete pancreatic juice, a mixture of products that includes protease and bicarbonate. The receptor for secretin is a G-protein coupled receptor that is expressed not only in the pancreas, but also in the brain. Recent reports indicate that secretin alleviates the symptoms of autism in some patients. However, there are conflicting reports regarding secretins effects. To analyze the mechanisms between secretin signaling and the development of autism, we generated the secretin receptor deficient mice. The mutant mice appeared normal; however, they had social behavioral abnormalities. In addition, we observed a significant reduction in hippocampal long-term potentiation (LTP) induction following high frequency stimulation and throughout the induction and maintenance phases of LTP. These results suggest that secretin signaling may be involved in normal neuronal and social behavioral development. Secretin receptor deficient mice may provide valuable new perspectives on diseases of autism.

Molecular investigation of SNPs previously associated with Parkinson disease (PD) risk. *M.A. Nouredine¹, J.M. van der Walt¹, M.A. Hauser¹, E.R. Martin¹, J. Pearson², M.A. Garcia-Blanco², J.M. Vance¹.* 1) Center for Human Genetics, Duke Univ. Medical Ctr, Durham, NC; 2) Duke University Medical Center, Durham, NC.

PD is marked by death of dopaminergic neurons (DN) within the substantia nigra. Current research supports the involvement of fibroblast growth factor 20 (FGF20) in the survival of DNs. We have recently reported a strong association between PD and FGF20 in a large family-based study. SNPs rs1989754 (C allele, $p=0.0006$) and ss20399075 (T allele, $p=0.0008$) are both positively associated with PD risk and exist in strong linkage disequilibrium (LD). Stratified and haplotype analyses suggest that rs1989754C does not have an independent effect on risk; however, its effect is observed in the presence of ss20399075T. Molecular strategies are needed to identify the causal SNP in this association. We predicted that rs1989754 lies within a site (CGTG) required for binding of the hypoxia-induced factor Hif1-. We hypothesized that FGF20 expression may be regulated under hypoxia, and that rs1989754 may affect this expression. To test this hypothesis, we first examined whether FGF20 transcription is regulated by hypoxia. We demonstrate here that FGF20 transcript level increases two fold in PC12 cells grown under hypoxic conditions. We also show that the sequence containing and flanking rs1989754 can function as a binding site for Hif1- only in the presence the G allele (protective) but not the C allele (risk). Finally, we use cell culture-based luciferase reporter system to investigate if this potential Hif1- binding site is hypoxia-responsive in vivo and if this response is affected by the allele difference at rs1989754. Our initial results suggest that this site (either G or C at rs1989754) is not hypoxia responsive, which argues against the involvement of this potential Hif1- binding site in mediating the hypoxia response observed in FGF20. In conclusion, we provide the first evidence that FGF20 is hypoxia responsive and demonstrate molecular support for our association results that suggest rs1989754 is not a causal variant and that the association of rs1989754 with PD was due to strong LD with ss20399075. We intend to investigate the role of this SNP in the regulation of FGF20.

Failure to confirm association between AKT1 haplotype and schizophrenia in a Japanese case-control

population. *T. Ohtsuki*¹, *T. Inada*², *T. Arinami*¹. 1) Department of Medical Genetics, University of Tsukuba, Tsukuba, Ibaraki, Japan; 2) Department of Psychiatry and Psychobiology, Nagoya University, Graduate School of Medicine, Nagoya, Aichi, Japan.

AKT1 is a serine/threonine kinase known as protein kinase B. Glycogen synthase kinase 3 (GSK3) is one of the substrates of AKT1 and AKT1/GSK3 signaling is known as a target of lithium. AKT/ GSK-3 signaling pathway might be relevant to dopamine-related disorders. Recently, Emamian et al identified AKT1 as a potential schizophrenia susceptibility gene in families of northern European origin. Levels of the AKT1 protein and phosphorylation of GSK3 at Ser9 in lymphocytes and frontal cortex were significantly lower in patients with schizophrenia in comparison with the controls. In multi-SNP haplotype analysis, haplotypes in the AKT1 gene were associated with schizophrenia. Significant association between schizophrenia and an AKT1 haplotype associated with lower AKT1 protein level was shown. In this study, we attempted to confirm the association between AKT1 haplotypes and schizophrenia in Japanese subjects comprising of 559 patients with schizophrenia and 567 controls. We genotyped 5 SNPs using TaqMan assay. Tests for single-marker allelic association were performed by chi-square analysis. Haplotype was estimated with an expectation-maximization (EM) algorithm. Tests for haplotypic association were performed with COCAPHASE software. Pair-wise linkage disequilibrium (LD) was calculated with GOLD software. We observed no significant allelic or haplotypic association with schizophrenia. There may be several reasons such as the different ethnical background and familial loading, why we could not confirm the association between AKT1 haplotype and schizophrenia in our Japanese samples. Furthermore, we can not exclude the existence of a weak association because the 95% confidence interval of the odds ratio was 0.76-1.38 in our population. Written informed consent was obtained from all subjects. This study was approved by Ethics Committee of each authors affiliation.

Haplotype and association studies of variations in -Synuclein gene among Parkinson's disease population. A.

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We detected an association between a dinucleotide repeat (SNCA) in -Synuclein gene and sporadic Parkinsons disease (PD, Parsian et al., 1998). To replicate our previous finding in a larger sample and further determine the role of -Synuclein in the development of PD, we screened a sample of 226 familial PD, 353 sporadic PD, and 130 controls with the SNCA marker and 3 SNPs (770, int3, and int4) in the gene. There was Hardy-Weinberg disequilibria only with SNP int3 that was excluded in the haplotype analysis. There was also significant difference in allele frequency between African American and American Indian groups for SNCA marker ($p=0.03$). These two samples were excluded from further analysis because of sample size. Comparison of allele frequency differences between PD and controls for single-locus was significant only for SNCA ($p= 0.014$). This result was further shown via haplotype analysis of SNCA-int4 markers. One of the interesting results of our haplotype analyses is with inclusion/exclusion of SNP int3. The global case control association was not significant when the SNP int3 was included in four loci haplotype analysis. Otherwise, two and three loci haplotpes comparisons were significant. The explanation is that inclusion of locus in Hardy-Weinberg disequilibria in haplotype analysis will reduce power as shown by Epstein and Satten (2003). Our results indicate that SNCA locus may be in linkage disequilibrium with a mutation in the gene or itself could be a risk factor for PD.

Genetic study of LRP2/Megalin gene variants in autistic disorder. *I. Ricci¹, M. D'Amelio¹, R. Militerni², C. Bravaccio², S. Trillo³, F. Montecchi³, M. Elia⁴, C. Schneider⁵, R. Melmed⁵, T. Pascucci⁶, S. Puglisi-Allegra⁶, L. D'Agruma⁷, K.L. Reichelt⁸, A.M. Persico¹.* 1) Mol Psychiatry & Neurogenetics, Univ. Campus Bio-Medico, Rome, Italy; 2) Child Neuropsychiatry, II Univ. of Naples, Italy; 3) Child Neuropsychiatry, IRCCS Osp. Bambino-Gesú, Rome, Italy; 4) Neurology Serv., IRCCS Oasi Maria S.S., Troina (EN), Italy; 5) S.A.R.C., Phoenix, AZ; 6) Dept. of Psychology, Univ. La Sapienza, Rome, Italy; 6) Dept. of Psychology, Univ. La Sapienza, Rome, Italy; 7) Med. Genet. Serv., IRCCS Casa Sollievo dalla Sofferenza, S.Giovanni Rotondo (FG), Italy; 8) Dept. of Pediatric Res., Rikshospitalet, Univ. of Oslo, Norway.

Subgroups of autistic patients consistently display alterations in specific biochemical and morphological parameters (i.e., endophenotypes), including enhanced urinary oligopeptide excretion rates. This biochemical trait is present in up to 60% of autistic children compared to age- and sex-matched controls. Oligopeptides, 3-10 aminoacids in length, are physiologically filtered by the renal glomeruli and undergo tubular reabsorption through an LDL receptor family member, the lipoprotein receptor-related protein 2 (LRP2) also known as megalin or gp330. Reduced LRP2 amounts and/or altered LRP2 function could thus explain the enhanced urinary excretion rates present in this subgroup of autistic patients. Interestingly, LRP2 plays also a relevant role in human brain development particularly during the first trimester of pregnancy, by exerting the placental uptake of maternal LDL cholesterol. In addition, the LRP2 gene is located on human ch 2q31-q32.2, within a region linked to autism in several genome scans. We are in the process of genotyping eight SNPs spanning the LRP2 locus in 223 simplex and 28 multiplex families with primary autistic probands and in 186 controls. Initial assessments of two SNPs located in exons 3 and 54 currently yield non-significant case-control and intra-familial association trends. Supported by Telethon-Italy (GPP02019), the Fondation Jerome Lejeune, and the Cure Autism Now Foundation.

Investigation of the DAOA/G30 locus in panic disorder. *J. Schumacher*¹, *R. Abou Jamra*¹, *T. Becker*², *N. Klopp*³, *P. Franke*⁴, *C. Jacob*⁵, *P. Sand*⁵, *J. Fritze*⁶, *S. Ohlraun*⁷, *T.G. Schulze*⁷, *M. Rietschel*⁷, *T. Illig*³, *P. Propping*¹, *S. Cichon*⁸, *J. Deckert*⁹, *M.M. Noethen*⁸. 1) Institute of Human Genetics, University of Bonn, Germany; 2) Institute for Medical Biometry, Informatics and Epidemiology, University of Bonn, Germany; 3) Genome Analysis Center (GAC), GSF-National Research Center for Environment and Health, Neuherberg, Germany; 4) Department of Psychiatry, University of Bonn, Germany; 5) Department of Psychiatry, University of Würzburg, Germany; 6) Department of Psychiatry, University of Frankfurt, Germany; 7) Central Institute of Mental Health, Division Genetic Epidemiology in Psychiatry, Mannheim, Germany; 8) Life & Brain Center, University of Bonn, Germany; 9) Department of Psychiatry, University of Münster, Germany.

The DAOA/G30 locus on chromosome 13q33 can be considered a strong positional and functional candidate for panic disorder (PD). In the present study, we assessed whether genetic variation at this locus contributes to the development of PD using a case-control association study. We analyzed five SNPs in a German sample of 152 patients and 208 controls. In the single-locus analyses, three of five tested SNPs were significantly associated with PD at the $p < 0.05$ level (allelic odds ratios range between 1.35 and 1.39, genotypic odds ratios range between 1.84 and 2.12). Further support was obtained at the haplotypic level, suggesting risk-conferring as well as protective haplotypes. A stringent adjustment for testing multiple loci through a robust permutation procedure yielded an empirical p -value of 0.11 for the single marker analysis, suggesting the possibility of a chance finding. However, given that we have previously observed the very same alleles and haplotypes to be associated with disease in bipolar affective disorder and schizophrenia in the German population, we believe that our findings are of importance and warrant further replication. Our results suggest that DAOA/G30 contributes to psychiatric disease beyond diagnostic boundaries. It will be a matter for future studies to fully elucidate the phenotypic expression of the DAOA/G30 locus.

Genetic analysis of glutamatergic signalling genes in alcohol dependence. *G. Schumann¹, M. Soyka², D. Rujescu², F. Matsuda³, A. Szegedi⁴, S. Wellek¹, N. Dahmen⁵, U. Preuss², M. Lathrop³, A. Heinz⁴, F. Henn¹, K. Mann¹.* 1) Central Inst. of Mental Health, Mannheim, Germany; 2) Dept. of Psychiatry, University of Munich; 3) Centre National de Gnotypage, Evry, France; 4) Dept. of Psychiatry, Humboldt University, Berlin; 5) Dept. of Psychiatry, University of Mainz.

Ethanol-induced glutamatergic signal transduction has been shown to influence pathophysiological mechanisms central to the development of alcohol dependence, including tolerance, withdrawal symptoms, craving, relapse and ethanol-related neurotoxicity. Ethanol acts specifically by inhibiting ionotropic N-methyl-D-aspartate (NMDA) receptors. Glutamatergic activation of NMDA-receptors initiates a Ca-mediated signal transduction cascade, which activates Calmodulin-dependent kinase (CamK) and the Ras pathway, leading to activation of the transcription factor CREB. Other proteins activated by NMDA receptors via PSD 95 include neuronal nitric oxide synthase (nNOS) and its effector GMP-kinaseII as well as Phosphatidyl Inositol Kinase 3 and the MAP kinase pathway. The goal of the present study is to systematically analyse genetic variations of NMDA-receptor subtypes and functionally related signal transduction genes which are known to be involved in glutamatergic neurotransmission in ethanol dependence in a large sample of German patients with alcohol dependence and unrelated controls. We attempted to include those NMDA-related genes where evidence for an alteration of alcohol drinking behaviour has been given in behavioural tests using knock-out mice. To this end we identified 10 genes involved in glutamatergic signal transduction and performed a SNP-discovery programme by sequencing analysis of the regulatory domains, exons and exon-intron boundaries of each gene. Next we performed haplotype analyses and genotyped those SNPs which account for the 95% most frequent haplotypes in a sample of 600 patients with alcohol dependence and 500 controls. Genotype-phenotype analysis with particular emphasis on oligogenic interactions was performed using a classical regression analysis. The results of this project will be presented.

***Pain1*, A MAJOR QUANTITATIVE TRAIT LOCUS FOR CHRONIC PAIN IN THE MOUSE, IS ALSO**

ASSOCIATED WITH CHRONIC PAIN LEVELS IN HUMANS AND RATS. Z. Seltzer^{1,2}, R. Dorfman³, E. Gershon², Y. Lu¹, Y. Blech-Hermoni¹, J. Livneh^{2,7}, Y. Shir^{4,5}, J-J. Vatine⁶, I. Sabsovich¹, T. Peretz⁵, M. Devor², R. Pfeiffer⁷. 1) University of Toronto, ON, Canada; 2) Hebrew University, Jerusalem, Israel; 3) Hospital for Sick Children, Toronto, ON, Canada; 4) McGill University, Montreal, QC, Canada; 5) Hadassah University Hospital, Jerusalem, Israel; 6) Reuth Medical Center, Tel Aviv, Israel; 7) Sheba Medical Center, Tel Hashomer, Israel.

Nerve injury produces chronic pain in some, but not all humans. Similar variability in chronic pain is also seen in rodents. We reported localizing a quantitative trait locus (QTL) in mouse chromosome 15 (at marker D15Mit28) that has a major effect on chronic pain following neurectomy. In the present study we examined whether this QTL, named *Pain1*, plays a role in chronic pain of rats and humans as well. Using 15 microsatellite markers we genotyped chromosome 7q13 in 45 HA and 37 LA rats, a region orthologous to mouse *Pain1*. Six markers on other chromosomes were genotyped as controls. HA rats were selectively bred from Sabra strain rats to express high levels, and LA rats to express low levels of chronic pain following nerve injury. We found that 6 of the 15 markers on 7q13 and 2 of the 6 control markers were dimorphic in the tested DNA samples. Significant linkage disequilibrium was found between the 2 rat lines and markers on 7q13, peaking at D7Got98 ($p < 0.000$), but not with the control markers. We then genotyped the orthologous regions of *Pain1* on human chromosomes 8q24 and 22q12-13 in 2 cohorts: women postmastectomy (N=650) and men and women following leg amputation (N=250), 55-80% of whom developed chronic pain. Significant association with chronic pain was found for microsatellite markers on 22q12-13 ($p < 0.01$), but not on 8q24. Then we genotyped 22q12-13 with a dense array of haplotyping SNPs and minimized the region associated with chronic pain to 50 kbp ($p < 0.002$). These results indicate that: (1) this rodent model for chronic pain is relevant for the study of pain genetics in humans, and (2) a gene at *Pain1* confers risk for chronic pain, making it an attractive target for further genetic studies in human chronic pain syndromes of various etiologies.

Glucocerebrosidase mutations in autopsy samples from subjects with parkinsonian syndromes. *E. Sidransky¹, J. Nguyen¹, J. Walker¹, O. Goker-Alpan¹, H.I. Hurtig², J.Q. Trojanowski³, B. Giasson⁴.* 1) NSB, NIMH/NHGRI, NIH, Bethesda, MD; 2) Dept. Neurology, U. Penn; 3) Dept. Path. & Lab. Med., U. Penn; 4) Dept. Pharmacology, U. Penn.; Philadelphia, PA.

An association between Gaucher disease (GD) and Parkinson disease (PD) has been demonstrated by the identification of parkinsonism in some patients with GD and among Gaucher carriers. Recently, screening of 57 brain bank samples from subjects with sporadic PD identified mutations in the glucocerebrosidase gene (GBA) in 17%. To better define the clinical and pathologic features among subjects carrying GBA mutations, we genotyped 23 brain samples from unrelated donors autopsied at the University of Pennsylvania. The samples included 9 cases with the pathologic diagnosis of Lewy Body Variant Alzheimer Disease (LBVAD), 4 with Diffuse Lewy Body Disease (DLB) and 10 with PD. Sequencing of all exons of GBA revealed that 5 subjects were heterozygous for mutations and 3 subjects carried the E326K alteration. Two of the 9 subjects with LBVAD had N370S and 1 carried I161N. The other 2 mutant alleles, A359X and T267I + E326K, were found among the 5 subjects with DLB, and E326K was found in 2 of the 10 subjects with PD. The age at death of the 23 subjects ranged from 58-97 years. The 7 subjects identified with GBA alterations were among the subjects with a younger age at death. Seven additional samples with the diagnosis of Multiple System Atrophy (MSA) were also screened, but no GBA alterations were identified.

This study confirms our previous observation that GBA mutations are observed with increased frequency in subjects with parkinsonism, but demonstrates that many do not have classic Parkinson disease. This finding supports the theory that mutant GBA protein may contribute to the formation of the insoluble aggregates seen in a gamut of neurodegenerative conformational disorders and may serve as a risk factor for the development of parkinsonian syndromes. By unraveling the relationship between altered GBA protein and parkinsonism, we may advance our understanding of the genetics, pathogenesis and treatment of different forms of parkinsonism.

Lack of linkage, association, or mutation with EKN1 and dyslexia in a colorado twin cohort. *S.D. Smith¹, H. Meng², J. Kenyon¹, M. Held², K. Hager³, J.C. DeFries⁴, B.F. Pennington⁵, R.K. Olson⁴, G.P. Page⁶, J.R. Gruen².* 1) Munroe Meyer Institute, Univ of Nebraska Medical Center, Omaha; 2) Department of Pediatrics, Yale Child Health Research Center, Yale Univ, New Haven; 3) WM Keck Foundation Biotechnology Resource Laboratory, Yale Univ, New Haven; 4) University of Colorado, Boulder; 5) University of Denver, Denver; 6) Department of Biostatistics, University of Alabama at Birmingham, Birmingham.

A candidate gene, EKN1, was recently described in a cohort of dyslexics from Finland for the dyslexia locus, DYX1 on chromosome 15q (Taipale et al, 2003, PNAS, 100(20): 11553). In this report a (2;15)(q11;21) translocation disrupting EKN1 segregated with dyslexia in a two-generation family, and one sequence polymorphism in the 5' untranslated region and a missense mutation showed significant association in 99 dyslexics compared to 195 controls ($p=0.006$ and 0.02). To confirm these results we interrogated the same polymorphisms in a cohort of 150 nuclear families with dyslexia ascertained through the Colorado Learning Disabilities Research Center (Boulder). We found a similar prevalence for the two polymorphisms, but could not confirm linkage nor transmission disequilibrium. Furthermore, we did not find any additional mutations in the 10 exons of EKN1 sequenced in at least 2 severely dyslexic subjects from 3 extended families with suggestive linkage to chromosome 15 markers ($LOD>1.0$). We conclude that while mutations of EKN1 may contribute to genetic susceptibility for dyslexia in the Finnish families reported, the effect was not seen in our cohort of predominantly European-American white families.

Molecular Studies on DYX1C1, a candidate gene for dyslexia. *I. Tapia-Paez¹, N. Kaminen², H. Anthoni¹, M. Peyrard-Janvid¹, J. Kere^{1,2}.* 1) Dept. of Biosciences at Novum, Karolinska Institute, Huddinge, Sweden; 2) Dept. of Medical Genetics, Biomedicum, University of Helsinki, Finland.

Dyslexia is a complex disorder characterized by reading disability despite normal intelligence and education; it affects 5-10% of the population. Genetic studies have repeatedly pointed several loci linked to dyslexia in chromosomes 2, 3, 6, 11, 15 and 18. Despite the efforts, only one gene has been cloned, DYX1C1 in chromosome 15 (Taipale et al, PNAS 2003 100(20): 11553-8). This gene was identified through studies in a Finnish family in which the translocation t(2;15) (q11;q21) cosegregated coincidentally with dyslexia. Two sequence changes in DYX1C1 displayed association with dyslexia in other families. One introduces a stop codon truncating the protein by four amino acids. The second change is in the 5UTR, close to the translation initiation site, this change affects the putative DNA binding site for several transcription factors and the Kozak sequence. To understand possible functional consequences of this change, we prepared constructs to study the binding of proteins in EMSA assays. Allele specific differential retardation of mobility was observed with two cell and nuclear extracts, suggesting that the dyslexia-associated polymorphism encompasses a site for nuclear factor binding. Studies are underway to identify the differentially binding protein(s). In another approach, we are studying the co-localisation of DYX1C1, p23, hsp90 and hsp72 in gene transfection models. Comparison of DYX1C1 from human and other nonhuman primates revealed differences in the sequence both in the coding and non-coding regions. Interestingly the changes are clustered in exon 5. We have prepared constructs with these changes and are currently investigating their possible differential processing in a neuroblastoma cell line.

Association of Catechol-o-methyltransferase with Schizophrenia in North American mixed and Portuguese populations. *J.M. Trakalo¹, J. Cui¹, T. Klempan¹, K. Schindler², M.H. Azevedo², C. Pato², M. Pato², F. Macciardi¹, J.L. Kennedy¹.* 1) Psychiatric Neurogenetics Section, Centre for Addiction and Mental Health, Toronto, Canada; 2) Centre for Psychiatric and Molecular Genetics, SUNY Upstate Medical University and Behavioural Health Care Line, Syracuse, New York, USA.

Catechol-o-methyltransferase (COMT) is a catalytic enzyme responsible for the metabolism of dopamine and other catecholamines. The importance of the role of COMT in dopaminergic neurotransmission, coupled with its location on chromosome 22q11, a region associated with psychosis, has made COMT a continued target of association studies of schizophrenia, although these studies to date have failed to produce a definitive conclusion regarding the role of COMT in schizophrenia. The focus of many of these studies is a functional polymorphism, Val158Met, responsible for a 3-4 fold reduction in COMT activity. In this study, we examined the Val158Met polymorphism, along with two other polymorphisms, an A/G substitution at position -287 and a C-Insertion in the 3'UTR, for association with schizophrenia. 114 triad families from a mixed population collected in Toronto, Canada, and 156 multiplex families collected from mainland Portugal and the Azores Islands were genotyped for all three markers and analyzed using the TDT-STDT, FBAT, and Transmit programs. No significant associations were detected using the TDT approach; however, a significant association was detected for the -287A/G polymorphism in the Toronto population with FBAT ($z=2.009$, $p=0.044$). In addition, a significant association was detected in the Portuguese sample for the 3'UTR polymorphism ($z=2.246$, $p=0.025$). No significant associations were detected for the Val158Met polymorphism under either of these methods. Finally, haplotype analyses using Transmit revealed a significant association in the Portuguese population for two common haplotypes ($p=0.0058$ and $p=0.0192$). Together, these results support a role for COMT in the aetiology of schizophrenia; however, it appears at present that the alleles responsible do not include the Val158Met polymorphism. Further studies including more markers and increased sample size are warranted in order to clarify the current findings.

Association between a T/C polymorphism of the CYP46 intron 2 and the late-onset Alzheimers disease. *L. Wang¹, B. Wang¹, F. Jin¹, Z. Yang², C. Zhang¹, Z. Liu³, C. Zheng³, S. Ueda⁴.* 1) Human & Animal Genetics, Inst Gen, Chinese Academy Sci, Beijing, China; 2) Laboratory for Medical Genetics, Institute of Geriatrics, Beijing hospital, Ministry of Health, Beijing 100730, China; 3) Jiangbin Hospital, Nanning, Guangxi 530021, China; 4) Dept. of Biological Science, Graduate School of Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.

We analyzed 99 sporadic late-onset Alzheimers disease (LOAD) Han Chinese patients and 113 healthy Han Chinese as controls, in order to investigate possible involvement of the cholesterol 24-hydroxylase (CYP46) gene (T/C in intron 2) and apolipoprotein E (APOE) gene polymorphisms in the manifestation of LOAD. We found an obvious association between CYP46 TT genotype and LOAD (OR=2.98, 95 percent, CI 1.64-5.44, P0.001). A clear increase of the risk to develop AD was also observed in subjects carrying both the CYP46 TT genotype and the APOE 4-allele (OR=12.94, 95 percent, CI 4.26-39.32, P0.001). Our data reveal that not only CYP46 TT homozygotes but also T allele carriers have an increased risk for LOAD in Chinese population. Our data reveal that CYP46 TT homozygots and T allele carriers have an increased risk for LOAD in Chinese population, and suggest a strong synergetic interaction between CYP46 TT genotype and APOE 4 carrier status.

Gene Expression Profiles in APOE Isoform-Specific Hippocampus of Alzheimer Disease (AD) Using Serial Analysis of Gene Expression (SAGE) and Long-SAGE. *P-T. Xu, Y-J. Li, C. Kroner, X-J. Qin, A. Green-Odlum, C. Hulette, D. Schmechel, J. Ervin, M. Hauser, M. Pericak-Vance, J. Gilbert.* Dept Medicine & Human Genetics, Duke Univ Medical Ctr, Durham, NC.

There is overwhelming evidence that multiple genes play an important role in Alzheimer disease (AD). Serial analysis of gene expression (SAGE) and Long-SAGE are powerful techniques for the generation of quantitative information about tissue specific gene expression. To identify new genes conferring susceptibility to Alzheimer disease, and study the influence of APOE genotype on gene expression in AD, we have generated four SAGE libraries using human brain hippocampal RNA isolated from AD patients with APOE3/3, APOE3/4 and APOE4/4 alleles, and a normal control (APOE3/3). We have also generated two Long-SAGE libraries using human brain hippocampal RNA isolated from an AD patient with APOE3/3 allele and a non-demented control (APOE3/3). Quantitation of the number of times a particular tag is observed provides the patterns of gene expression in APOE allele specific AD and non-demented control hippocampal samples. SAGE and LongSAGE library construction was performed with 10 ug total RNA according to the standard SAGE protocol. All the SAGE and Long-SAGE libraries were estimated to contain more than 480,000 tags (>14,400 clones). We have sequenced 2,500 clones of APOE3/3 control SAGE library and 2,500 clones of APOE3/3 AD SAGE library using ABI 3700 automated sequencer. Analyses of sequence files of these two libraries using the eSAGE software demonstrate 78,125 tags extracted from APOE3/3 AD library and 70,456 tags extracted from APOE3/3 control. A total of 37,899 transcripts are identified. Preliminary analysis indicates that 351 genes are more than 5 fold differentially expressed in AD patients and normal control with $p < 0.05$. In AD patients, 148 genes were significantly up-regulated and 203 genes were down-regulated compared with normal control. Through the combined use of both Affymetrix microarray and SAGE, we have obtained a detailed and quantitative APOE isoform-specific gene expression profile of known and/or novel new genes conferring susceptibility to Alzheimer disease.

PR missense variants: a possible association with puerperal psychosis. *J. Yan¹, J. Feng¹, J. Chen¹, C. Katz¹, I.R. Jones², N. Craddock², S.S. Sommer¹.* 1) Dept Molecular Genetics, City of Hope, Duarte, CA; 2) Dept Psychological Medicine, University of Wales College of Medicine, UK.

Steroid and thyroid hormone administration can alter behavior; in occasional individuals, psychosis can result. The levels of progesterone and other hormones change dramatically after birth, suggesting that mutations in the steroid receptor gene family may predispose to puerperal psychosis. To investigate this possibility, the progesterone receptor gene was scanned by DOVAM-S (Detection of Virtually All Mutations-SSCP) in 146 patients with puerperal psychosis. Seven missense variants, three silent changes and two intronic changes were identified. Three missense variants at highly conserved amino acids were found in nine patients with puerperal psychosis while none were found in 100 ethnically matched healthy controls (P=0.008). A possible association between PR and puerperal psychosis may warrant further study.

Association Study of the Myelin Oligodendrocyte Glycoprotein (MOG) Gene in Obsessive-Compulsive Disorder and Schizophrenia. *G.C.M. Zai¹, N. King¹, P. Arnold¹, E. Burroughs², G.W.H. Wong¹, W.G. Honer⁴, C.L. Barr³, M.A.*

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Obsessive-compulsive disorder (OCD) and schizophrenia (SCZ) are serious neuropsychiatric disorders and might involve autoimmune processes. The human leukocyte antigen (HLA) system has been implicated in several genetic studies of SCZ, whereas a subgroup of children with OCD are believed to develop symptoms in response to Group A beta-hemolytic streptococcal infections. The myelin oligodendrocyte glycoprotein (MOG) gene, which is located close to the HLA region on chromosome 6p, is considered a candidate for OCD and SCZ due to its association with white matter abnormalities and its role in immune processes, particularly in mediating the complement cascade. Four polymorphisms in the MOG gene, (CA)_n, (TAAA)_n, and two intronic polymorphisms, C1334T and C10991T, were investigated for the possibility of association with OCD using 159 OCD probands and their families, with SCZ using 111 SCZ probands and their families, and 182 SCZ case-control samples (matched with age, gender, and ethnicity). We investigated the transmission of alleles/haplotypes of these four MOG polymorphisms using the transmission disequilibrium test and TRANSMIT TDT haplotype test in OCD and SCZ, family-based association test (FBAT) in OCD, Kruskal-Wallis Test and case-control analysis in SCZ. FBAT analysis of MOG-(TAAA)_n showed positive association with OCD (allele 2, P=0.02) and with quantitative symptom severity (P=0.020). The haplotype 1.2.2.13 of MOG (P=0.01) was also significantly associated with OCD. However, we did not observe biased transmission of alleles/haplotypes or significant differences between the allelic/genotype distributions of SCZ cases and healthy controls. Our results suggest that MOG is not associated with SCZ, but may play a role in OCD.

Association of PNMT promoter haplotypes with blood pressure and venodilation response to isoproterenol. X.

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Blunted vasodilator responses to agonists have been implicated in the pathogenesis of hypertension, but the identity of the contributing genes, especially the genetic variants, remain largely unknown. Phenylethanolamine N-Methyltransferase (PNMT) is the terminal enzyme in the synthesis of epinephrine, the primary endogenous α -agonist. To investigate the role of PNMT in the development of hypertension and the mechanisms involved, we examined the associations of PNMT promoter haplotype with blood pressure and dorsal hand vein dilation response to α -agonist isoproterenol. Two single nucleotide polymorphisms (SNPs) at promoter 353 (A/G) and 148 (A/G) were genotyped in 218 white subjects. By using a maximum likelihood method, all 4 possible haplotypes were found in our population: AA (55.5%), GG (31%), AG (12.4%), and GA (1.1%). Subjects with haplotype AG had significantly higher diastolic but not systolic blood pressure (782 mmHg vs. 741 mmHg for other haplotypes, $p=0.04$), which was even more significant in subjects with diplotype AG/AG (883 mmHg vs. 762 mmHg) and in the younger group (age<45, $p=0.004$). Dorsal hand vein response to ISO, as measured by ultrasound, was nearly completely blunted in subjects with haplotype AG (0.920% vs. 322% for Haplotype AA and 424% for Haplotype GG, $p<0.001$ by ANOVA, $n=88$). In addition, Haplotype AG was associated with increased brain PNMT activity by 15% ($p=0.03$), elevated plasma epinephrine level by 80% ($P=0.03$), and decreased lymphocyte α_2 -adrenergic receptor sensitivity by 40% ($p=0.04$) when compared with other haplotypes. In conclusion, PNMT promoter haplotypes appear to correlate with PNMT enzymatic activity, epinephrine production and α_2 -adrenergic receptor function, and thus can be used to predict the venodilation response to α -agonists as well as individual variation in blood pressure.

Complexities of molecular diagnosis for ventricular arrhythmias. *L. Baumbach, T. Miller, H. Travers, L. You, M.E. Ahearn, W. Lanier, E. Estrella, S. Elliott, R. Myerburg, N. Bishopric.* Univ Miami Sch Medicine, Miami, FL.

Molecular diagnosis of Long QT syndrome (LQTS) and related arrhythmogenic disorders remains highly complex, due to numerous private mutations, variants of unknown significance, and decreased penetrance alleles. We report our initial laboratory experience performing mutation screens of five LQTS disease genes (KVLQT1, KCNH2, KCNE1, KCEN2, SCN5A) in 34 consecutive probands referred from a tertiary care hospital for clinically suspected LQTS or Jervell-Lange-Nielsen syndrome (JLNS) (n=20), unexplained sudden cardiac death (SCD; n= 10), or pharmacologically-induced LQT and *torsade* (PI-LQT; n=4). Mutation screening was conducted using exon-specific PCR, SSCP, and DNA sequencing of all SSCP variants. DNA variants were reviewed for relative frequency and possible disease association using mutation databases and published literature. Presumed disease-causing mutations were detected in seven (39%) suspected LQTS patients. Five of these were in KVLQT1; two were in KCNH2. Five were novel (71.4%). Coinheritance of these alterations with disease presence was proven in 6/7 families analyzed. It is of interest that two of the five cases with KVLQT1 mutations had a second mutation (D85N) in KCNE1, the modifying subunit of KVLQT1. A number of synonymous and non-synonymous SNPs were also detected in all genes. In SCD patients, two (20%) carried pathogenic mutations (one in KCNH2, one in SCN5A), while the remaining patients displayed numerous SNPs in all five genes, including at least one SCN5A variant in each patient. Of the four PI-LQT patients, only one had a known pathogenic mutation (KVLQT1, G189R), while two had rare and/or novel SNPs (KCNH2 1809 C>T; KVLQT1 I93I + SCN5A G3183A). These results suggest that with increased availability of genetic testing for LQTS, *bona fide* disease-causing mutations are likely to be identified in fewer than 50% of all patients referred with suspected LQTS, and that the majority of patients will harbor novel and/or common gene variants of unknown significance. Additional functional and epidemiological studies are warranted to establish better genotype-phenotype correlations in LQTS.

Identification of Three Novel Mutations in MEF2A Associated with Coronary Artery Disease and Myocardial Infarction. *MR. Krishna Bhagavatula*^{1,2,3}, *Chun. Fan*^{1,2}, *June. Cassano*², *Edward.F. Plow*^{1,2}, *Eric.J. Topol*^{1,2}, *Qing. Wang*^{1,2,3}. 1) MolecularCardiology,LRI; 2) CenterforCardiovascularGenetics,CCF; 3) Dept of BGES,CSU.

Background: Coronary artery disease (CAD) and myocardial infarction (MI) are the leading cause of deaths in the U.S. We have recently established MEF2A, encoding a transcription factor in the endothelium of coronary arteries, as the first gene responsible for autosomal dominant CAD/MI gene (Science, 2003; 302: 1578-1581), and a 21-bp deletion of MEF2A was identified in a large family with CAD/MI. However, the prevalence of MEF2A mutations in the CAD/MI population is unknown. Here we report the results from mutational analysis of MEF2A in 200 independent patients with CAD/MI (males <55 years and females <60 years of age) and 200 individuals with normal angiograms. **Methods:** Mutational analysis was carried out using SSCP and DNA sequence analyses. The functional consequence of the newly-identified MEF2A mutations were examined using a transcription activation assay with the ANF-700p-luciferase reporter gene and transient transfection of wild type or mutant MEF2A proteins in HeLa cells. **Results:** Three novel mutations were identified in exon 7 of MEF2A in four of 200 CAD/MI patients (2%), and none of the mutations were detected in 200 normal individuals. These mutations include N263S identified in two independent CAD/MI patients, P279L in one patient, and G283D in another patient. Analysis of family members revealed that the father of the patient with mutation P279L also carried the mutation and has the diagnosis of CAD. The three mutations are located within or close to the major transcription activation domain of MEF2A (amino acids 274-373), and significantly reduced the transcription activation activity of MEF2A. These results suggest that N263S, P279L, and G283D are functional mutations. **Conclusions :** These results provide the first confirmatory evidence of our previous report that MEF2A mutations cause CAD and MI and indicate that a significant percent of the CAD/MI population (2%) may carry mutations in MEF2A. Further definition of the prevalence of MEF2A mutations is clearly warranted.

Association of the STRK1 gene PDE4D with Stroke in a U.S. Population. *V.H. Brophy¹, S.K. Ro¹, A.M. Valdes¹, S. Gretarsdottir⁴, G. Thorleifsson⁴, L. Lui², J. Mangacat¹, N. Umblas¹, L.G. Bentley¹, J. Li¹, S. Cheng¹, J.R. Gulcher⁴, K. Stefansson⁴, S.R. Cummings³, W.S. Browner³, H.A. Erlich¹.* 1) Roche Molecular Systems, Alameda, CA; 2) University of California, San Francisco, CA; 3) California Pacific Medical Center, San Francisco, CA; 4) deCODE Genetics, Reykjavik, Iceland.

Phosphodiesterase 4D (PDE4D) underlies the STRK1 linkage peak on chromosome 5q12 identified in Iceland for stroke. We genotyped 13 single-nucleotide polymorphisms (SNPs) and one microsatellite in a nested case-control sample of elderly white women (>65 years) from the Study of Osteoporotic Fractures (SOF) in the U.S. The fourteen polymorphisms lie in two large regions of the 1.5-Mb gene, one at the 5 end and one in the middle. The 249 women who suffered an ischemic stroke during an average of 5.4 years of follow-up were compared to 587 controls. Two SNPs showed borderline or significant association with stroke in recessive models: SNP222 (hazards ratio [HR] 0.60, P=0.05) and SNP42 (HR 1.35 P=0.09). (In the Icelandic population, the relative risks (RR) were 0.88, P = 0.11 and 0.67, P = 0.01 respectively, for additive models.) Adjustment for age, hypertension, diabetes, smoking, and weight also yielded borderline significance for SNP219 and SNP220 for SOF stroke. Additionally, we found that four of the SNPs were associated with hypertension in all samples (P<0.05) and SNP175 was associated with hypertension in the control samples (P = 0.006, OR 1.65). Estimated haplotype frequencies suggested association of a 5-SNP haplotype at the 5 end of the gene (AACAG, OR 0.68, P=0.06) while a 6-SNP haplotype had an OR of 1.91 (ACTATG, P=0.02). These differed from the stroke-associated Icelandic haplotypes of the same SNPs at one site each: AACAA, (RR 1.8, P=0.00002) and ACCATG (RR 1.5, P=0.0004, unpub.) respectively. Differing LD patterns within these large haplotypes and with the causal variant(s) may explain the variability in associations between the two populations. These data are consistent with an association of the PDE4D gene and stroke in a non-Icelandic population.

An association study supports *COL24A1* as a modifier gene in human heart failure. *K.M. Carlson*¹, *B.R. Pedersen*², *M.P. Donahue*², *E.R. Hauser*², *W.E. Kraus*², *H.A. Rockman*², *D.A. Marchuk*¹. 1) Molecular Genetics and Microbiology, Duke University, Durham, NC; 2) Medicine, Duke University, Durham, NC.

Modifier genes play an important role in the pathogenesis of heart failure (HF). The use of animal models of HF is one approach to identifying modifier genes whose role in human HF can be studied. Using this approach, we previously identified Collagen Type XXIV, alpha 1 (*COL24A1*) as a candidate gene for *Hrtfm2* (Heart failure modifier 2) in the Calsequestrin transgenic mouse model of HF. In order to confirm its role as a modifier of human HF, we identified 11 missense SNPs in *COL24A1* by sequencing the gene in 16 human HF patients. Preliminary analysis identified a suggestive association between one SNP, A61V, and ejection fraction (EF) in a small subset of human HF patients (n=243). We now report a significant association between *COL24A1* and EF in a second, larger human population. Subjects from the CATHGEN sample of the Duke database for coronary artery disease were selected according to EF and their CAD index (CADi), a validated angiographical measure of the extent of coronary atherosclerosis that correlates with outcome. Our population consisted of 301 young cardiac patients (age < 55) and 168 older cardiac patients (age > 55) with reduced EF, and 204 elderly subjects (age > 60) with normal EF matched for the extent of CAD. The association of *COL24A1* with EF was tested using linear regression to examine the relationship between genotype and EF adjusting for covariates. By genotyping 5 *COL24A1* exon 3 missense SNPs in our entire population, we identified a significant association between EF and 3 *COL24A1* coding SNPs: V95M (p=0.0161), P151L (p=0.0218), and E340K (p=0.0137). An examination of mean EF by genotype revealed that each minor allele is associated with a decrease in EF in a gene dose dependent manner. We note that the allele frequencies of each variant are small. In conclusion, we identified *COL24A1* as a high target candidate gene for a modifier of HF using a mouse model of HF. We now demonstrate a significant association between 3 *COL24A1* SNPs and EF in a 968 patient population. These data support a role for *COL24A1* as a modifier gene of human HF.

Gender dependent association of thrombospondin-4 A387P polymorphism with myocardial infarction. *J. Cui*^{1,2}, *E. Randell*^{1, 2, 4}, *J. Renouf*⁴, *G. Sun*², *F-Y. Han*^{1, 2, 4}, *B. Younghusband*¹, *Y-G. Xie*^{1, 2, 3, 4}. 1) Dept. of Genetics; 2) Dept. of Lab Med; 3) Dept. of Pediatrics, Memorial University, St. John's, NL, Canada; 4) Program of Lab Med, Health Care Corporation of St. John's, NL, Canada.

Background - A recently identified novel missense variant of Thrombospondin-4 (TSP4), A389P (29926 GC) was shown to have a possible proatherogenic effect based on function studies. Only a few genetic association studies have been published and with contradictory results. We have been taking advantage of the genetically isolated population of Newfoundland to study the impact of a variety of gene variants on risk for myocardial infarction (MI). As part of this study we tested the hypothesis that the TSP-4 gene variant is a risk factor for MI. The Newfoundland population is a genetically isolated population, which allows us to avoid genetic admixture, a commonly faced challenge in association studies. **Methods and Results** We performed a case control study in 500 patients with MI and 500 normal control individuals from the Newfoundland population. Genotyping of TSP4, A389P (29926 GC) was conducted using real-time PCR. The gene frequency of TSP-4 A387P in the Newfoundland population was determined to be 23.1% which is similar to that reported using other Caucasian populations elsewhere. Slightly increased carrier frequency, allele frequency and frequency of homozygosity for the 387P allele were observed in patients with MI compared with controls (43.6% vs. 41.8%, 24.9% vs. 23.1% and 6.2% vs. 4.4% respectively), but the differences did not reach statistical significance. However, when the distribution of the TSP-4 A387P variant was further analyzed by sub-grouping patients and controls according to age and sex, an increased prevalence of the 387P allele was observed in female patients with MI compared with the sex matched controls (OR = 1.34, 0.93 to 1.94). Furthermore, homozygosity for the 387P allele was significantly associated with female patients compared with the female controls (OR = 2.96, 1.29 to 6.78, P=0.008). **Conclusions**- We conclude that the TSP-4 A387P polymorphism is associated with MI in females, especially when present in the homozygous state. This suggests a gene dosage effect.

APOE4 Allele is Associated with Poor Functional Outcome after Ischemic Stroke. *K. Furie^{1,4}, M. Sale², J. Stein^{3,4}, M. Ning^{1,4}, A. Muzikansky¹, R. Betensky⁵, S. Greenberg^{1,4}, B. Hyman^{1,4}, S. Rich², F. Hsu², P. Kelly^{1,6}.* 1) Massachusetts General Hospital, Boston MA; 2) Center for Human Genomics, Wake Forest Univ School of Medicine, Winston-Salem, NC; 3) Spaulding Rehabilitation Hospital, Boston MA; 4) Harvard Medical School, Boston MA; 5) Harvard School of Public Health, Boston MA; 6) Mater University Hospital, Dublin, Ireland.

Background: APOE4 allele, a susceptibility gene for Alzheimer's disease, is associated with a higher risk of recurrent stroke and poor functional outcome after other neurological insults. The relationship between APOE genotype and functional outcome after ischemic stroke is controversial. We hypothesized that the APOE4 allele would predict poor functional recovery following ischemic stroke. Methods: We enrolled consecutive ischemic stroke patients admitted to a single in-patient acute rehabilitation hospital. Stroke severity (NIHSS) and demographic data were obtained at baseline. Functional status (Functional Independence Measure [FIM] score) was determined at admission, discharge, and at 6-months. Standard RFLP methods using Hha I digest and high-resolution agarose electrophoresis of exon 4-derived PCR products were used for E2, E3 and E4 allele typing. Results: Of 252 patients enrolled, APOE genotypes were available on 214 subjects. The mean age was 66.7 years. 57% were male. The mean FIM was 6723.7 on admission, 9327.0 on discharge, and 10133.1 at 6 months. The mean change in FIM between admission and discharge was 25.517.2 and between admission and 6-month follow-up, 32.928.9. The genotype frequencies were E2/3 11.2%, E2/4 1.87%, E3/3 70.56%, E3/4 14.49%, E4/4 1.87%. In a multiple regression model, the APOE4 allele predicted FIM score at discharge, independent of age and baseline stroke severity ($p=0.0155$). Conclusion: In this sample, the APOE4 allele independently predicted functional outcome following ischemic stroke. This may be an important factor to consider in the design and interpretation of clinical trials in stroke recovery.

A multigenic approach for investigating the synergistic effects of Down syndrome congenital heart disease

candidate genes using the *Drosophila* heart. *T.R. Grossman*¹, *R.J. Wessells*², *G.M. Barlow*³, *R. Bodmer*², *J.R.*

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Down syndrome (DS) is a major cause of congenital heart disease (CHD). Molecular studies have established a candidate region that includes D21S55 (21q22.13) through the telomere. Potential candidate genes for DS-CHD were prioritized based on their expression in the heart and on homology to genes involved in AV cushion morphogenesis. These genes are: SH3BGR, DSCAM, WRB, Collagen VI A1/A2, Collagen XVIII and HES1. It has been suggested that trisomic inheritance of these gene(s) is sufficient to cause subsets of DS-CHD. We chose to use the *Drosophila* heart as an in vivo system to study the effect of mis-expression of the candidate genes in the heart in an attempt to identify which gene(s) are responsible for the DS-CHD phenotype. *Drosophila* is the invertebrate genetic model system most closely related to humans and is emerging as a powerful tool for analyzing the function of human disease genes. Key determinants of cardiogenesis in the fly also play fundamental roles in early cardiac specification in vertebrates. Using the UAS-GAL4 system we mis-expressed the human candidate genes and their fly counterparts in the fly heart. The effect of mis-expression of the candidate genes on the formation and physiology of the heart is being studied using sensitive physiological assays. In addition, we are examining potential synergistic interactions between the different candidate genes by mis-expressing them in the heart in each possible permutation, an approach that would not be practical in the mouse model system. Our preliminary results show that mis-expression of DS-CHD candidate genes can result in significant heart defects such as decreased heart rate and increased rate of stress-induced heart failure. We also show that the same genes that exhibit a significant heart defect result in strong visible phenotypes when mis-expressed in the eye or in the wing. Taken together these results indicate that mammalian DS-CHD genes are active in the fly heart and may interfere with its normal function and physiology.

5-Lipoxygenase Promoter Polymorphism is Associated with Myocardial Infarction, Elevated C-reactive Protein, and Fasting Insulin Levels. *J. Hartiala*¹, *E. Ipp*², *C. Tymchuk*¹, *A. Lusis*³, *M. Mehrabian*³, *T. de Bruin*⁴, *H. Campos*⁵, *H. Allayee*¹. 1) Dept of Prev Medicine, Univ Southern Calif, Los Angeles, CA; 2) Dept. of Medicine, Harbor-UCLA Med Cntr, Los Angeles, CA; 3) Dept. of Medicine, UCLA, Los Angeles, CA; 4) CardioVasc Res Inst, Maastrich Univ. the Netherlands; 5) Dept. of Nutrition, Harvard School of Public Health.

Coronary artery disease (CAD) is the most common cause of death in Western societies and develops as a result of the complex interaction between genetic and environmental factors. In previous studies with mice, we provided evidence that 5-lipoxygenase (5-LO) deficiency resulted in a profound resistance to aortic lesion development. By examining the human 5-LO gene, we further demonstrated that shorter (i.e. 3 and 4) repeats of a promoter polymorphism were associated with carotid atherosclerosis, C-reactive protein (CRP) levels, and fasting plasma insulin in a randomly ascertained population. To confirm these initial observations, we have now genotyped the same polymorphism in two additional cohorts. In a group of 412 combined hyperlipidemic individuals, the frequency of the 4 allele was significantly higher in those who had suffered a myocardial infarction (MI) than those who had not (0.21 vs. 0.15; $P < .05$). Additionally, homozygosity for the 4 allele was associated with elevated fasting insulin ($P < .05$) in these individuals. In a second cohort consisting of MI patients ($n=1029$) and matched controls ($n=1096$), there was no significant difference in the frequency of the 4 allele, perhaps due to genetic heterogeneity and/or population differences. However, control individuals in this cohort who were homozygous for the 4 allele had significantly elevated levels of plasma CRP (3.9mg/L vs. 2.8mg/L; $P < .04$) and fasting insulin (21mEq/L vs. 15mEq/L; $P < .03$) compared to control individuals with other genotypes. These associations with CRP and insulin were not observed in the MI group, perhaps due to the physiological and inflammatory effects of suffering an MI. Taken together, these findings confirm our initial observations and provide support for the pleiotropic role of 5-LO in CAD and characteristics of the metabolic syndrome in humans.

Polymorphism in ornithine carbamoyltransferase (OTC) is associated with hypertension related phenotypes.

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OTC is a nuclear gene encoding a mitochondrial enzyme. If deficient it causes an inborn error of urea cycle metabolism. Here we present an association study between OTC, hypertension (HT) and related phenotypes among subjects from French Canadian families. An ATT insertion/deletion in the intron 5 of OTC gene with the frequency of 31/69% was used. The sample consisted of 774 individuals from 115 families with HT and dyslipidemia. All blood pressure (BP) measurements were higher among gen1 males and females (1/1+1/2) comparing to gen2 carriers. The difference was strong among HT but not among normotensive (NT) with the highest differences for sleep (mmHg),: systolic, diastolic, mean arterial BP and for heart rate : 12.5, 6.9, 10.2 and 6.4 (p from 0.005 to 0.001), after correction for age, gender and years of HT. Only in males a difference in SBP was observed in response to standing (higher increase in males). While gen1 was similarly associated with BP increase in both genders, an opposite effect for some phenotypes was observed. Gen1 males had higher Na excretion by 29 mg/min, p=0.04 while females had lower by 32.8, p=0.001 and also females had lower Na concentration by 21.0 mg/dl p=0.003 in the urine, after correction for urine volume, age and body size. These differences were not accompanied by difference in aldosterone level, but gen1 males had lower renin change after water load (0.10 vs. 0.51ng Al/ml/hr, p=0.02). Also gen1 males had higher lvm (left ventricle mass) by 30.1g, p=0.05 while females had lower lvm: 39.1, p=0.03. In qualitative analysis a strong dimorphism was detected for late onset HT with odds ratio, OR=10.3 (p=0.01) for gen2 males and OR=3.4(p=0.03) for gen1 females. In conclusion: OTC gene is involved in hypertension and some related metabolic phenotypes in French Canadians with a strong gender dimorphism in sodium metabolism, heart hypertrophy and in predisposition to late onset HT.

Genetic Variations of ETS1 Gene Associated with Hypertension in Japanese. *H. Morisaki¹, I. Yamanaka¹, A. Takashima¹, Y. Kokubo², H. Tomoike³, T. Morisaki^{1,4}.* 1) Dept Bioscience, NCVC Research Inst, Suita, Osaka, Japan; 2) Dept Preventive Cardiology, NCVC, Suita, Osaka, Japan; 3) NCVC Hospital, Suita, Osaka, Japan; 4) Dept Molecular Pathophysiology, Osaka U Grad Sch Pharm Sci, Suita, Osaka, Japan.

ETS1 is an important transcription factor widely involved in many aspects of vascular development, remodeling, and atherosclerotic changes, which are important features of hypertensive pathophysiology. We selected 5 SNPs in the ETS1 gene locus as locus-representing markers and conducted a large-scale association study using consecutive Suita Study samples in Japan, comprised of 1014 women and 866 men, to evaluate the genetic effects of ETS1 on the hypertensive phenotype. Logistic regression analysis showed that 2 SNPs in the promoter region, (-1015)2G/3G and (-606)A/T, were significantly associated with the hypertensive phenotype in men and women, respectively. (-606)T-allele frequency was significantly higher than that of (-606)A-allele in hypertensive cases ($p=0.00004$), and (-606)T carriers were significantly associated with the hypertensive phenotype in females after adjusting for age, BMI, smoking, alcohol consumption, and history of hyperlipidemia or diabetes ($p=0.003$, OR 1.73). For females over 60 years old, the adjusted OR increased to 1.87. ANCOVA analysis using samples from non-medicated subjects also showed that SBP and DBP were significantly higher among (-606)T carriers than non-carriers. Further, male (-1015)3G carriers showed a significant association with the normotensive controls ($p=0.0149$, OR 0.60) and (-1015)3G alleles were significantly less frequent than (-1015)2G alleles in hypertensive cases, suggesting that these may have a protective effect on the hypertensive phenotype. Haplotype analysis revealed that the (-1015)2G/(-606)A haplotype was significantly less frequent in hypertensive females. Our results show that common genetic variations in promoters of the ETS1 gene are associated with the hypertensive phenotype in Japanese.

Genome-wide association study to identify genes related to myocardial infarction and their functional analyses.

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Myocardial infarction, one of the leading causes of death in the world, is characterized by abrupt occlusion of coronary artery resulting in irreversible damage to cardiac muscle. A number of molecules have been reported to be possibly involved in its pathogenesis, including those that mediate inflammation. Lymphotoxin- (LTA) is one of the earliest cytokines produced in vascular inflammatory processes, and it has been recently shown that functional variations in LTA are associated with susceptibility to myocardial infarction by a large-scale genetic association study using single nucleotide polymorphism (SNP). Here we show that LTA binds to galectin-2, a member of galactose binding lectin family, whose function is not well characterized. Case-control association study revealed a SNP in *LGALS2* encoding galectin-2 was also associated with susceptibility to myocardial infarction. This genetic change was shown to affect transcriptional level of galectin-2, which led to altered secretion of LTA possibly by mediating its intracellular trafficking through binding to microtubules, thereby affecting the degree of inflammation. Both proteins were shown to be expressed in the smooth muscle cells (SMCs) and macrophages in the human atherosclerotic lesions. Our findings implicate the importance of LTA cascade in the pathogenesis of myocardial infarction.

ZASP and Melusin variants are Associated with Dilated Cardiomyopathy and Ventricular Dilation Secondary to Hypertension. V. Palumbo¹, A. Belgrano¹, A. Gatti¹, S. Crovella¹, A. Amoroso¹, M. Vatta^{2,1}. 1) Department of Reproductive and Developmental, University of Trieste, Trieste, Trieste, Italy; 2) Department of Pediatrics (Cardiology), Baylor College of Medicine, Houston, Texas, USA.

ZASP is a striated muscle protein essential for maintaining the structural integrity of the Z-line during biomechanical stress. Recently, ZASP mutations have been identified in dilated cardiomyopathy (DCM). Melusin is a striate muscle protein that interacts with the Integrin 1 cytoplasmic domain and localizes to the costameres. Melusin plays a crucial role in the hypertrophic response to mechanical overload. Melusin-null mice, if subjected to pressure overload develop cardiac remodeling and DCM. We investigated the role of ZASP and Melusin in DCM and ventricular dilation secondary to pressure overload due to primary hypertension. We screened 100 DCM and 100 probands with essential hypertension and secondary DCM for mutations in ZASP and Melusin. We identified the C302T missense mutations in ZASP (P101L residue change) in one DCM proband, and the G511A mutation in one patient with secondary DCM (A171T aminoacid substitution). In addition, two synonymous single nucleotide polymorphisms (SNP) were found in exonic and intronic sequences throughout the gene. Two novel Melusin variants were detected in secondary DCM: a synonymous SNP (C872T) that affects the consensus motif of an exonic splicing enhancer (ESE) and segregates with the cardiac phenotype in the proband's family, and a 7 bp intronic duplication in close proximity of exon 6 5'-splice site (IVS6+12_18dupTTTTGAG) in a sporadic case. All ZASP and Melusin variants were absent in 200 control chromosomes. Secondary structure prediction suggests abnormal protein in ZASP mutants, and altered splicing for Melusin variants. We hypothesize that ZASP variants affects the Z-line structural integrity during pressure overload and chronic mechanical stress. Melusin, unlike ZASP, does not appear to play a role in the sarcomeric structural organization; rather it seems to act as biochemical sensor during chronic cytoskeletal mechanical tension as it occurs in ventricular dilation secondary to hypertension.

Promoter Polymorphisms of Heme Oxygenase 1 and Their Influence on Cardiovascular Disease in African Americans. *J. Pohorence Ferguson, Y. Liu-Stratton, G.E. Cooke, P.F. Binkley.* Division of Cardiovascular Med, OSU Davis HLRI, Columbus, OH.

Heme oxygenase-1 (HO-1) is upregulated by stimuli that produce reactive oxygen species, and plays a protective role in cardiovascular diseases involving oxidative stress. A loss of HO-1 activity increases tissue susceptibility to oxidative damage and has been associated with the evolution of cardiovascular disease in animal models and human subjects. The transcriptional activity of HO-1 is reduced by a promoter microsatellite polymorphism, while a T(-413)A polymorphism in the promoter has been found to increase HO-1 transcriptional activity. Sequence analysis was performed on DNA extracted from a total of 377 subjects, 166 normal controls and 211 patients with cardiovascular disease, to examine the frequency of these polymorphisms. In the case of the microsatellite polymorphism, an allele was classified as L if the forward sequence (GT) repeat was 24 or the reverse sequence (AC) repeat was 27. The L allele was found to occur more frequently in African American subjects than Caucasian subjects, with 77% of African Americans having the L allele versus 55% of Caucasians ($p = 0.0002$). Examining the frequency of the T(-413)A polymorphism revealed that 98% of the subjects with an A at position 413 also had an L allele in the microsatellite polymorphism ($p = 0.0001$). When the population was divided by race, all African American subjects possessing an A at 413 also had the L allele ($p = 0.0004$). These findings are significant because they show that the African American population contains two seemingly contradictory polymorphisms in the promoter of the HO-1 enzyme. Further research is necessary to examine how the presence of both polymorphisms influences HO-1 transcription, and how this affects the development of cardiovascular disease in the African American population.

Genetic variation in COL6A1 in Down Syndrome individuals with congenital heart defects and their parents.

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Approximately 40% of individuals with Down syndrome (DS) are born with a congenital heart defect (CHD) and atrioventricular septal defects (AVSD) are the most common of those. Collagen VI, a heterotrimeric protein, is expressed in fetal and adult heart. The Collagen 6A1 gene (COL6A1) is located within a region of chromosome 21 implicated in the causation of DS-associated CHD and alterations in COL6A1 function or expression could negatively affect heart septation. Using a case (DS with AVSD) /control (DS without CHD) approach, we examined the association of genetic variation in the COL6A1 gene with the development of AVSD in DS individuals. Three haplotype blocks (5' block spanning exons 1-5, middle block spanning exons 5-20, 3' block spanning exons 21-35) tagged by 5 SNPs were identified from Patil et al (Patil et al, 2001, Science 294:1719-1722). Genotype frequencies in these 5 SNPs were then examined in 52 DS individuals with AVSD, 73 DS individuals without CHD, and all respective parents. Analyses were done separately for Caucasians(C) and African-Americans (AA). Comparison of frequencies for the two SNPs capturing the 3' block revealed significant differences between cases and controls (C $p=0.008$, $p=0.008$ / AA $p=0.018$, $p=0.004$) and between the respective Caucasian parents (C $p=0.016$, $p=0.005$). Significant differences were also noted between cases and controls, and parents for the 5' block tagged SNP in AA and in the middle block tagged SNP in C. Additionally we genotyped 4 coding SNPs (1 in exon 19 and 3 in exon 35) and found no significant difference between any of the study groups. Re-sequencing of 50 individuals of either population has failed to identify SNPs with >10% frequency in the coding portion of the triple helical region of the gene. This study supports previous reports describing increased genetic variation in COL6A1 in DS individuals with CHD.

***GATA4* Mutations in Patients with Congenital Heart Disease.** A. Tomita-Mitchell¹, D. McElhinney², C. Maslen³, V. Garg⁴, D. Srivastava⁴, E. Goldmuntz¹. 1) The Children's Hospital of Philadelphia, Philadelphia, PA; 2) The Children's Hospital, Boston, MA; 3) OHSU, Portland, OR; 4) UT Southwestern, Dallas, TX.

A recent report has implicated mutations in the transcription factor *GATA4* as a cause of familial cardiac septal defects. Mutations in *NKX2.5*, a molecular partner of *GATA4*, have been identified in familial and sporadic non-syndromic congenital heart disease (CHD), including septal and conotruncal defects. We hypothesized that *GATA4* mutations would be found in additional subjects with septal or related conotruncal defects. Our CHD patient cohort included: Tetralogy of Fallot (TOF, 201), ventricular septal defects (VSD, 137), atrial septal defects (ASD, 124), D-transposition of the great arteries (76), double outlet right ventricle (45), truncus arteriosus (20), interrupted aortic arch (11), L-transposition of the great arteries (10), and other malformations (3). Conotruncal and VSD patients tested negative for 22q11 deletions. We evaluated the *GATA4* coding region and exon-intron boundaries for sequence variants in our cohort by Denaturing HPLC (n=503) or Conformation Sensitive Gel Electrophoresis (n=124). Samples showing peak or band shifts were reamplified from genomic DNA and sequenced.

Four non-synonymous mutations (G93A, Q316E, A411V, D425N) were identified in five patients (ASD, 2; VSD, 2; TOF, 1) and were not seen in a control population of 88 individuals by direct sequencing. All four affected amino acid residues are conserved across species and two of the mutations lead to changes in polarity. Nine synonymous mutations were also identified in fifteen patients and were not observed in the control population.

In summary, we identified four non-synonymous variants in five of 627 subjects. Our data suggest these four variants could be disease related but additional functional studies are required. It appears that disease-related *GATA4* mutations occur in a small fraction of CHD patients and that *GATA4* mutations may be etiologically important in conotruncal as well as septal malformations.

Systematic search for SNPs within candidate genes for essential hypertension in a high-risk Chinese population.

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Essential hypertension has become a primary risk for premature death. Since 1999 we had started to investigate a relatively isolated population in Zhangwu, a rural county in northeast China, where an unusually high prevalence of the disease (40.0% in males and 32.0% in females, n=5208) was discovered. Following blood sampling a systematic study for single nucleotide polymorphisms (SNPs) in candidate genes including endothelial Na channel beta-subunit (beta-ENAC), G-protein beta3-subunit (GNB3), the beta-adrenoceptor gene (beta-AR) family, and With-no-lysine=K 4 (WNK4) was performed. So far we have excluded association between SNPs in the exon12 of beta-ENAC including T594M, which was studied extensively in Africans. As for GNB3, although our data did not support it a susceptible gene in the whole population, statistics did suggest that an 825C/T polymorphism may play an important role in the pathogenesis in females (n=133, control=257, P<0.05). For the beta-AR gene family, four SNPs have been studied (beta1-AR: Arg389Gly and Gly49Ser; beta2-AR: Arg16Gly; beta3-AR: Trp64Arg), among which the first two were strongly associated with the disease (n=144, control=174, P=0.0348 and P=0.0000, respectively). On expression analysis, cytoplasmic cAMP of homozygotes for Arg389 and Ser49 were significantly higher than those of homozygotes for Gly389 or Gly49. Screening for SNPs in WNK4, in particular Ala589Ser in exon8 has found a strong association between a T allele and onset of hypertension (n=259, control=235, P<0.05). As a missense mutation, the Ala589Ser polymorphism in exon8 may diminish the function of WNK4 kinase through substitution of a nonpolar residue alanine with a polar residue serine. Our further work will include analysis of more SNPs (and haplotype(s)) and study of structural and functional significance of particular genotypes.

Proteomics Analysis of L-6TG Cell Line Constitutively Expressed Pantothenate Kinase 4 (PANK4). *Y. Li¹, Q. Feng¹, M. Xiong², J. Zuo¹, Y. Meng¹, F. Fang¹*. 1) National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Peking Union Medical College, Chinese Academy of Medical Sciences, 5# Dong Dan San Tiao, Beijing, P. R. China 100005; 2) Human Genetics Center, The University of Texas, Health Science Center at Houston, Houston TX 77030, USA.

A novel gene, pantothenate kinase 4 (PanK4), was cloned from the SD rat skeleton muscle stimulated by high concentration glucose. PanK4 had been identified to interact with the M2-type pyruvate kinase (Pkm2), which is a key enzyme in the glycolysis pathway. The result showed that PanK4 might involve in the blood glucose control. In order to find more factors that might be affected by PanK4, the proteomics analysis of myoblast cell line L-6TG constitutively expressed PanK4 protein was carried out. From the 2-D gel result, there are 100 hundreds protein spots existing in experimental groups but not in the control groups. By the MALDI-TOF-MS analysis to these spots, heat shock 70kD protein 8, 58 kD glucose regulated protein, Muscle specific enolase and Gamma-actin were identified. The result showed that PanK4 might involve in the Akt/PKB pathway, Jat/Stat pathway: IL-6 receptor family, glycolysis and cell skeleton activation. PanK4 might be a very important factor in regulating the insulin pathway, blood glucose control and some genes transcription.

ALK4: A Possible Role in Neoplasia in Ulcerative Colitis. *S. Mahid*¹, *M. Fox*¹, *D. Colliver*¹, *W. Zacharias*², *S. Galandiuk*¹. 1) Dept. of Surgery, Price Institute of Surgical Research, University of Louisville, Louisville, KY; 2) Brown Cancer Center, University of Louisville, KY.

Background: Inflammatory Bowel Diseases consist of a group of poorly understood disorders including Crohns disease (CD) and ulcerative colitis (UC). Both disorders are associated with an increased risk of colorectal cancer. While numerous IBD susceptibility loci have been characterized, none have been linked with neoplastic progression. Methods: Affymetrix U95 Av2 arrays were used. mRNA was obtained from benign UC, UC dysplasia and UC colon cancer in 3 individuals each who had all 3 tissue types. Transcripts that were present were fit using a repeated measure design across tissue types using SAS Proc Mixed (SAS, Inc.) Results: 699 transcripts were differentially expressed in UC dysplasia compared to benign UC; this was statistically different in 132. Gene profiles in development of dysplasia largely differed from those in progression from dysplasia to cancer. Prior case control studies in our population confirmed an association with IBD susceptibility locus 2 on chromosome 12q. Interestingly, this was the site of one of the statistically differentially expressed genes in dysplasia: ALK4 Least Squares Means, Overall Means: Benign 411, Dysplasia 862.9, Cancer 661.1. Conclusion: ALK4 a TGF- superfamily receptor and tumor suppressor gene is located on 12q3, within the IBD 2 locus. Paradoxically, it was over expressed in dysplasia as compared to benign UC. Upon activation with activin, ALK-4 receptor complexes and phosphorylates Smad 2, which in turn complexes with Smad 4 and may activate or repress the transcription of various genes. We believe that ALK-4 may play a role in neoplastic progression in UC despite its role as a tumor suppressor gene.

Small nucleolar RNAs HBII-52 at chromosome 15q11.2 display a high density of SNPs in autistic patients. M.

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Chromosome 15q11-q13 has been suggested as a candidate region for autism, based on whole genome screens and linkage disequilibrium studies. In addition, cytogenetic abnormalities at the 15q11-q13 locus are relatively frequent in autistic patients. Several brain-specific, paternally imprinted small nucleolar RNAs (snoRNAs) genes with unknown function have been identified at chromosome 15q11.2. HBII-52 snoRNAs are of particular interest since they might be involved in post-transcriptional modifications of the serotonin 2C receptor mRNA. HBII-52 snoRNAs are located within segmental duplications and are present in multiple copies with an elevated sequence identity. Gene conversion and duplicon-mediated rearrangements might occur in this region and therefore could lead to clinical consequences. In Spanish autistic patients, we analysed by SSCP and sequencing of 25 of the 47 HBII-52 snoRNA genes (~6250 bp) searching for either SNPs or de novo gene conversion events. We have detected 20 SNPs and a microdeletion in 20 chromosomes analyzed. The study of the parental chromosomes has ruled out de novo gene conversion events. Given the small number of chromosomes analyzed, the observed SNP density (~3.36/1 kb) is strikingly high. Interestingly, three patients displayed minor alleles for multiple SNPs (>5) in the analyzed region. These SNPs might represent paralogous sequence variants and might be indicative for partial duplications of the HBII-52 region. The study of larger sample of autistic patients and control individuals should allow us to evaluate if accumulation of multiple SNP alleles or possible duplications of HBII-52 snoRNAs region is indeed relevant to autistic disorder.

I/D Polymorphism of angiotensin-Converting Enzyme (ACE) gene in a population with morbid obesity in Spain.
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BACKGROUND:The renin-angiotensin system (RAS) plays a role in the pathogenesis of metabolic diseases. This system is expressed in human adipose tissue, where angiotensin II stimulates the production of prostacyclin, which affects adipogenesis and adipocyte metabolism. The human ACE gene contains a polymorphism consisting of the presence (I) or absence (D) of a 287-bp in intron 16 of the ACE gene. Genotype DD patients have higher plasma and tissue concentrations of ACE. We investigated the association of the I/D polymorphism of the ACE gene with the presence of morbid obesity in a sample of a population from Spain. **MATERIALS AND METHODS:**We performed a complete familial and personal history from 30 patients with morbid obesity (BMI > 35). We collected the following data: Birth weight, age of obesity onset (apparent causing effect) speed of obesity progression, actual weight and maximum weight. We also asked the patients about their nutritional habits (compulsive and anxious eating) previous dieting (type, duration, results), medication, co-morbidities and related treatments. The physical exam included size, weight, BMI, body fat distribution, and regular biochemical parameters. **RESULTS:**The ACE polymorphism was detected by polymerase chain reaction (PCR) amplification, following the Genomica kit protocol. The genotype DD was found in 80% of the morbid obesity population, the ID in 10% and the genotype II in 10% of patients, respectively. The allele frequencies was 85% for the D allele and 15% for I allele. The data corresponding to a control population of 108 volunteers were: DD 38%, ID 50 % and II 12%, with and allele frequency of 63% D and 37% I. The statistical analysis shows a significant difference ($p < 0.05$) between the frequency of the DD genotype in the morbidly obese patients and the control subjects. **CONCLUSIONS:** These results suggest that the genotype DD could be considered as a risk factor for morbid obesity in this sample population from Spain.

Gender Difference in the Genetics of ADHD. *L. Park*¹, *K. Nummy*², *M. Rappley*⁴, *J. Nigg*³, *K. Friderici*². 1) Genetics Program; 2) Microbiology and Molecular Genetics; 3) Department of Psychology; 4) Peds/Hum. Dev., Michigan State University, East Lansing, MI.

ADHD is a prevalent, costly, impairing, chronic psychiatric disorder originating in childhood and showing high heritability. Molecular genetic studies of it have generally overlooked potentially crucial gender differences in genetic susceptibility. Like many other psychiatric disorders, ADHD clearly differs by gender in prevalence, at least in childhood, and apparently also in symptom expression and clinical correlates. Two probable models could explain the greater incidence of ADHD in boys: the polygenic multiple threshold model and the constitutional variability model. The former posits that girls require more genetic risk factors than boys to be affected; the latter suggests that different causal factors affect girls and boys differently. Our association tests grouping girls and boys separately using eight polymorphisms on three candidate genes for ADHD strongly support the constitutional variability model in the gender differences observed in ADHD. The dopamine transporter (SLC6A3) and the α -2A-adrenergic receptor gene (ADRA2A) were related to ADHD significantly in girls, whereas the dopamine receptor D4 (DRD4) was related to ADHD significantly in boys. This result might be explained by gender differences in the dosage sensitivity of the catecholamine system identified previously, which is derived from gender different expressions of those genes and differential susceptibility of dopaminergic system in dopa-responsive dystonia. These candidate genes may reflect different causal factors between genders for ADHD. However, the possibility cannot be excluded that the similarity of basic family genetic background predisposes to ADHD leading to common etiology between genders. Our genetic data also support this by showing the association for two of the genes, ADRA2A and DRD4, are in the same direction for both genders. The genetic difference between genders in ADHD seems to be important yet questionable in the etiology of ADHD. Taken together our data demonstrate that genetic susceptibility to ADHD is regulated differently in girls and boys.

HLA B44 is associated with decreased severity of autoimmune lymphoproliferative syndrome in individuals with CD95 mutations. *M.M. Vacek¹, A.A. Schäffer², J. Davis¹, R.E. Fischer¹, J.K. Dale³, S.E. Straus³, J.M. Puck¹.* 1) Genetics and Molecular Biology Branch, NHGRI/NIH, Bethesda, MD; 2) Computational Biology Branch, NCBI/NIH, Bethesda, MD; 3) Laboratory of Clinical Infectious Disease, NIAID/NIH, Bethesda, MD.

Autoimmune lymphoproliferative syndrome (ALPS) is a disorder of lymphocyte apoptosis characterized by nonmalignant lymphadenopathy and splenomegaly, expansion of T cells lacking CD4 or CD8 surface markers and increased incidence of autoimmune diseases and lymphoma. Most patients with ALPS have dominant, heterozygous mutations in the tumor necrosis factor receptor superfamily member 6 gene (TNFRSF6), which encodes CD95, also known as Fas, a mediator of apoptosis signaling. The specific Fas mutation a person carries influences the severity of disease. Mutations causing disruption of the intracellular death domain of the CD95 molecule impair apoptosis the most and are associated with the highest penetrance of ALPS features and the greatest morbidity in mutation-bearing relatives of affected probands. However, wide variation in the incidence and severity of clinical and laboratory features of ALPS is seen among members of pedigrees who share the same mutation, implying that additional factors modify the ALPS phenotype. To evaluate HLA as a candidate modifier locus we typed HLA A, B, DQB1, and DRB1 alleles in 317 individuals from 63 unrelated families with defined TNFRSF6 mutations associated with ALPS. We also developed a quantitative severity score and performed statistical analysis with TRANSMIT and FBAT. Among healthy mutation-bearing individuals, transmission of HLA-B44 was significantly overrepresented (nominal $P < 0.009$) as compared to transmission in patients with severe clinical features of ALPS. The B44 allele may exert a protective role in ALPS.

Region Specific Toxicity of Mutant Huntingtin in Human Huntingtons Disease Brain is Directly Replicated in YAC128 Mouse Model. *J.M. Van Raamsdonk^{1,2}, Z. Murphy², M.R. Hayden^{1,2}.* 1) Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 2) Centre for Molecular Medicine and Therapeutics, University of British Columbia, Vancouver, B.C., V5H 4H4, Canada.

Huntingtons disease (HD) is an age onset neurodegenerative disorder that primarily affects the striatum and cortex despite ubiquitous expression of the mutant huntingtin (htt) protein. The YAC128 mouse model of HD has been shown to recapitulate the behavioural abnormalities, cognitive deficits and neuropathology seen in human HD. Here we demonstrate region specific toxicity within the brain that corresponds to the regions affected in HD. At 12 months of age, YAC128 mice show decreased striatal volume (10.4 % decrease; WT = 12.1 0.1 mm³, N = 8; YAC128 = 10.8 0.2 mm³, N = 8; p < 0.001), decreased cortical volume (6.7% decrease; WT = 18.0 0.4 mm³, N = 8; YAC128 = 16.8 0.4 mm³, N = 8; p = 0.04) and decreased volume of the globus pallidus (10.8 % decrease; WT = 1.6 0.06 mm³, N = 8; YAC128 = 1.43 0.04 mm³, N = 8; p = 0.04). At this time point, the volume of the hippocampus (WT = 4.89 0.24 mm³, N = 8; YAC128 = 4.95 0.41 mm³, N = 8; p = 0.9) and the volume of the cerebellum (WT = 48.1 1.3 mm³, N = 5; YAC128 = 50.5 0.9 mm³, N = 5; p = 0.2) are unchanged suggesting that, as in humans, mutant htt has a preferential toxicity for the striatum, cortex and globus pallidus with less toxic manifestations in the hippocampus and cerebellum. We will also present data on the region specific expression levels of mutant and wild type htt protein within the brain and correlate htt expression to the observed pathology. Overall, the ability of YAC128 mice to accurately model human HD will allow us to gain insight into the region specific toxicity in human HD and supports the use of YAC128 mice in pre-clinical therapeutic trials for HD.

Microarray analysis of gene/transcript expression in Angelman syndrome: Deletion versus UPD. *D.C. Bittel¹, N. Kibiryeva¹, Z. Talebizadeh¹, D.J. Driscoll², M.G. Butler¹*. 1) Children's Mercy Hospitals and Clinics and University of Missouri-Kansas City School of Medicine, Kansas City, MO; 2) University of Florida, College of Medicine, Gainesville, FL.

Angelman syndrome (AS) is a neurodevelopmental disorder characterized by severe learning difficulties, ataxia, subtle dysmorphic facial features, and a happy disposition. AS and Prader-Willi syndrome (PWS) were the first examples of genomic imprinting in humans and result from genetic abnormalities of the chromosome 15q11-q13 region. AS results from functional defects in the *UBE3A* gene located within 15q11-q13. Defects in *UBE3A* can be caused by maternal deletion (65-75%), paternal uniparental disomy (UPD, 3-5%), imprinting defects (ID, 3-10%) or point mutations or small deletions within the *UBE3A* gene (5-10%). The remaining AS subjects have no identifiable molecular abnormality (10-14%). The genetic subtypes of AS have a number of phenotypic and behavioral differences. AS subjects with deletions tend to have the most severe phenotype. AS subjects with UPD or ID have a lower incidence of hypopigmentation, microcephaly, and seizures. To further our understanding of the complex nature of gene expression in the 15q11-q13 region and to identify candidate genes/transcripts which contribute to the variation in individuals with AS we have generated cDNA microarrays containing most of the genes/transcripts from the region. We compared gene expression from actively growing lymphoblastoid cell lines established from nine male subjects [6 with AS (3 with 15q deletion and 3 with uniparental paternal disomy 15 or UPD) and 3 controls]. Expression patterns were consistent with expectations for genes known to be biallelically expressed (e.g., *GAPD*, *FBN*), paternally expressed (e.g., *SNRPN*) or maternally expressed (e.g., *UBE3A*, *ATP10C*). Paternal or maternal allele expression bias was detected in several genes/transcripts in our study (e.g., *GABRA5*, *GABRB3*). Our results indicate subtle and unpredicted changes in gene expression which may explain phenotypic differences among genetic subtypes of AS. Furthermore, these results support our previously reported microarray gene expression studies in PWS.

Genes epistatic to *FMRI* involved in the autism behavioral phenotype. *S.T. Nowicki*^{1,2}, *L. Li*^{3,5}, *D.B. Milliken*¹, *J.P. Gregg*^{1,4}, *R.J. Hagerman*^{1,2}, *D.M. Rocke*⁵, *P.J. Hagerman*^{1,3}. 1) M.I.N.D. Institute, UCDMC, Sacramento, CA; 2) Div. of Developmental and Behavioral Pediatrics, Dept of Pediatrics, UCDMC, Sacramento, CA; 3) Dept of Biochemistry and Molecular Medicine, School of Medicine, UC Davis, Davis, CA; 4) Dept of Pathology, UCDMC, Sacramento, CA; 5) Dept of Applied Science, Div of Biostatistics, UC Davis, Davis, CA.

Fragile X syndrome (FXS) is a single-gene disorder with a strong association with autism (AUT). Approximately 33% of patients with FXS will have autism. Our hypothesis is that one or more genes, from a larger pool of autism susceptibility genes, are responsible for the autism phenotype associated with FXS. To test this hypothesis, we analyzed the gene expression profile of 27 patients divided in 2 groups (Group 1: 5 patients with FXS+AUT and 9 patients with FXS, Group 2: 5 patients with FXS+AUT and 8 patients with FXS). A cognitive assessment and ADOS-G were performed. Total RNA was isolated from whole blood. Samples were prepared and analyzed on the HG-U133A (Group 1) and the HG-U133Plus2.0 (Group 2) gene chips using standard protocols (Affymetrix). The data from each group was transformed and normalized as described by Geller et al. [(2003) *Bioinformatics* 19:1817]. A two-sample t-test was performed between the FXS+AUT and FXS samples for each group. There were 1,113 probes in common from both groups that were differentially expressed. The Ingenuity Pathway Analysis tool found 427 genes that were contained in 116 global networks that involved 926 total focus genes from the database. Several cellular mediators of neurotrophic factor signaling were differentially expressed including Cdc42, JNK, -catenin, JIP1/2/3, PP2A, MEK1/2, AKT, JAK1, JIP1, mTOR, PINCH1, and TSC1. Many of these intracellular signaling pathways are involved in axon outgrowth and guidance, and dendritic branching and arborization. The RT-PCR validation of these targets and the metabolic consequences will be discussed. These data support our hypothesis that differential gene expression in FXS patients with autism offers additional clues to identify epistatic genes that contribute to the development of autism.

Maternal genetic polymorphisms of folate pathway and risk of Down Syndrome in Campania, Italy. *I. Scala¹, M. Sellitto¹, B. Granese¹, S. Salomé¹, M. Cestari¹, A. Sammartino², A. Pepe¹, C. Nappi², G. Sebastio¹, P. Mastroiacovo³, G. Andria¹.* 1) Dept of Pediatrics, Federico II University, Naples, Italy; 2) Dept. of Gynecology and Obstetrics, Federico II University, Naples, Italy; 3) International Centre on Birth Defect, Rome, Italy.

Down syndrome (DS) is caused by trisomy of chromosome 21. The molecular mechanisms of non-disjunction are elusive. Genetic polymorphisms of folate pathway have been associated with DS in some studies, but not in others. We present a comprehensive study of polymorphisms of genes of the folate pathway in mothers as risk factors for DS in Campania, Italy. DNA from 84 DS mothers and from 233 controls from the same population was analysed for the following polymorphisms: methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C, methionine synthase reductase (MTRR) A66G, cystathionine-beta-synthase (CBS) 844ins68, methionine synthase (MTR) A2756G and reduced-folate carrier 1(RFC1) A80G by specific restriction enzymes. MTHFR 677TT, CBS 844ins68, MTRR 66GG and MTR 2756GG genotypes were not associated with increased risk of DS. Conversely, the MTHFR 1298CC and the RFC1 80GG genotypes were more frequent in DS mothers vs controls with a 2.29-fold increased risk (OR 2.29; 95% CI 0.98-5.37) and a 2.22-fold increased risk (OR 2.22; 95% CI 0.96-5.12) of DS, respectively. MTHFR 677TT and 1298CC genotypes were associated with maternal age at delivery. By stratifying the DS mothers' population by the age at delivery, we found a statistically significant association between mothers younger than 30 years old and MTHFR TT genotype (<25 years: OR 11.25; 95% CI 1.66-87.6; 25-29 years: OR 5.4; 95% CI 1.05-30.44). The MTHFR 1298CC genotype was associated with maternal age at delivery \geq 30 years (OR 4.49; 95% CI 0.9-43.3). The role of folate/homocysteine metabolism in DS is under evaluation in several settings with discordant results, due to three main critical issues: wide difference of allelic frequencies in various populations, choice of controls, study sample size. Well designed collaborative multicenter study and a comprehensive polymorphisms evaluation could help to address the issue.

Identification of disease genes using gene network models inferred from gene expression profiles. *D. di Bernardo*¹, *T.S. Gardner*², *J.J. Collins*². 1) Computational biology, TIGEM, Naples, Naples, Italy; 2) Department of Bioengineering, Boston University, Boston, MA.

Genetic diseases result from a hierarchical chain of events initiated by the mutation of a single gene and resulting in the dysregulation of several genes (secondary responders). We propose a computational approach to identify disease genes from whole genome gene expression profiles. The approach analyzes the expression profile of a mutated cell using a gene-network model to distinguish the mutated gene, responsible for the disease, from genes that exhibit only secondary responses. This approach was successful in *Saccharomyces cerevisiae* and it can be scaled up to mammalian organisms. Our approach first constructs a computational model of regulatory interactions in the organism. The model is learned using a training data set of whole-genome expression profiles resulting from a variety of treatments (knockouts, chemical agents, etc.). Second, we apply the model of regulatory interactions to the expression profile obtained from a yeast strain with a mutation in one of its genes. The model analyzes the mutant profile to suppress expression changes of genes that are due to internal regulatory influences (secondary responders) and amplifies the expression changes of the mutated gene. The result is a list of genes ranked by their likelihood of being the mutated gene. We tested our algorithm on a data set consisting of 300 whole-genome yeast expression profiles. We tested the ability of the algorithm to identify 11 genes with mutated promoters in the data set. The algorithm correctly identified the gene in 10 out of the 11 cases (91% correct). In contrast, expression-change ranking identified the correct gene for only 4 of the 11 mutations. We examined whether the algorithm could identify mutations that affect only protein activity, but not mRNA expression level. We applied the algorithm to a yeast strain carrying a mutation yielding a truncated protein and successfully identified the mutated gene. Our approach could be applied to mammalian organisms. Expression profile obtained by treating cells with compound libraries may serve as suitable data set for analysis by the algorithm.

Association of the 744TC polymorphism in the P2RY12 gene with response to clopidogrel following coronary stent implantation. *U. Cavallari¹, E. Trabetti¹, M. Biscuola¹, D.J. Angiolillo², A. Fernandez-Ortiz³, E. Bernardo³, C. Ramirez³, M. Sabate³, P. Jimenez-Quevedo³, R. Moreno³, R. Hernandez-Antolin³, C. Banuelos³, J. Escaned³, F. Alfonso³, M.A. Costa², T.A. Bass², C. Macaya³, P.F. Pignatti¹.* 1) Dept. Mother-Child & Biol-Genetics, Univ Verona, Verona, Italy; 2) Cardiovascular Center - Shands Jacksonville - Univ Florida, USA; 3) Cardiovascular Institute - San Carlos Univ Hospital, Madrid, Spain.

Clopidogrel inhibits the purinergic receptor P2Y, G-protein coupled, 12 (P2RY12). Recently, polymorphisms of this receptor have been associated with platelet aggregation and have been suggested to modulate the broad interindividual clopidogrel response. The 744TC polymorphism of the P2RY12 gene was assessed in a total of 116 pts undergoing elective coronary stenting: 36 pts receiving a standard 300 mg loading dose (Group A) and 80 pts on long-term clopidogrel (75 mg/d) treatment (Group B). Platelet function, including platelet aggregation (PA) and platelet activation, was assessed. PA was assessed by light transmittance aggregometry in platelet-rich plasma stimulated with ADP (6 M). Platelet activation was assessed by P-selectin expression and activated GPIIb/IIIa (anti-Fibrinogen-FITC) using whole blood flow cytometry in ADP (2 M)-stimulated platelets. Platelet function was assessed at baseline (pts only on aspirin) and 4 and 24 hours following clopidogrel loading dose in Group A and when pts were on clopidogrel treatment for at least 1 month in group B. Patients were similar for baseline demographic and clinical characteristics. No differences were observed between groups for any of the assessed platelet function profiles according to genotype. In conclusion, the 744TC polymorphism of the P2RY12 gene did not appear to be associated with platelet response to clopidogrel in this group of patients.

Replication study of PDE4D and ALOX5AP gene variants in a Scottish Stroke population. *S. Gretarsdottir¹, A. Helgadóttir¹, D. St.Clair², A. Manolescu¹, J. Cheung², G. Thorleifsson¹, A. Pasdar², U. Thorsteinsdóttir¹, S.F. Grant¹, A. Kong¹, J.R. Gulcher¹, K. Stefansson¹, M.J. MacLeod².* 1) deCODE Genetics, Reykjavik, Iceland; 2) Aberdeen Royal Infirmary and University of Aberdeen Medical School, Aberdeen, UK.

Genetic variations in two genes have recently been associated to common forms of stroke in Iceland, the Phosphodiesterase 4D gene (PDE4D) and 5-Lipoxygenase activating protein (ALOX5AP) (Gretarsdottir et al.2003, NatGen 35:131-8; Helgadóttir et al.2004, NatGen 36:233-9).To further assess the contribution of the PDE4D and ALOX5AP variants to stroke a replication case-control study was carried out in a Scottish stroke population (n=574 patients and n=718 controls).The majority of the Scottish patients had ischemic stroke.The replication was carried out by genotyping the previously identified PDE4D (at-risk and protective haplotype) and ALOX5AP (HapA and HapB).Comparison of the estimated haplotype frequency of the Icelandic ALOX5AP at-risk haplotype (HapA) between Scottish stroke patients and controls showed that the patients had a significantly higher frequency than controls (allelic frequency 18.5% versus 14.3%, respectively, p=0.007).The ALOX5AP variant called HapB, a variant found to be associated with myocardial infarction in British individuals,did not show association to stroke in this Scottish cohort.The Icelandic PDE4D protective haplotype was also significantly replicated in the Scottish stroke cohort (allelic frequency in patients 16.8% versus 20.5%in controls,p-value=0.0078).The Icelandic at-risk haplotype (defined by microsatellite AC008818-1 allele 0 and SNP 45 allele G)was not shown to confer risk of stroke.However by exchanging the microsatellite allele from 0 to 4 a significant difference was observed between Scottish patients and controls (allelic frequency 22.1%versus 17.7%,p=0.006).Taken together,our results point to an important role for both the ALOX5AP and PDE4D genes in the pathogenesis of common forms of stroke, not only in Icelanders but also in a population outside Iceland.

Genetic and functional evidence for the role of USF1 transcription factor in hyperlipidemias. *J. Naukkarinen¹, M. Gentile¹, A. Soro-Paavonen², J. Saarela¹, P. Pajukanta³, M-R. Taskinen², L. Peltonen^{1,3}.* 1) Department of Molecular Medicine, National Public Health Institute, Finland and Department of Medical Genetics, University of Helsinki, Biomedicum, 00290 Helsinki, Finland; 2) Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland; 3) Department of Human Genetics, David Geffen School of Medicine at UCLA, University of California, Los Angeles, California 90095-7088, USA.

Familial combined hyperlipidemia (FCHL) is the most common familial dyslipidemia in man. It is characterized by elevated levels of serum total cholesterol, triglycerides or both and is observed in about 20% of individuals with premature coronary heart disease.

We recently reported the discovery of a major gene behind FCHL on 1q23. Linkage and association was established to a transcription factor called USF1 representing an important regulator of numerous genes involved in lipid and glucose metabolism (Pajukanta et al. *Nature Genetics*, 2004). Component traits of FCHL overlap with the metabolic syndrome and type II diabetes suggesting that USF1 may contribute to the molecular background of these conditions as well. The linkage of the USF1 region on 1q23 to FCHL and type II diabetes has already been replicated in several populations and recently USF1 was found to be associated with glucose and lipid parameters in a European wide study sample (Putt et al. *Hum Mol Genet*. 2004)

Our analyses of expression profiles in fat biopsy samples from individuals with FCHL indicate differences in the expression level of the USF1 regulated genes conditional on the associated haplotype. The functional role of this DNA region was studied in vitro. A putative regulatory element identified in the associated haplotype was shown to enhance expression of a reporter gene and a 20bp DNA region was also shown to bind a factor from nuclear extract in a gel-shift experiment. Furthermore, a SNP within this 20bp segment seems to influence specificity of the binding, implying its functional importance in the dynamics of USF1 expression and hence in dyslipidemias.

Association between soluble guanylate cyclase (sGC) subunit gene, GUCY1A2, and hypertension in a black South African population. *M. Penny*¹, *S. Bentivegna*², *S. Lebejko*², *L. Wood*², *G. Wimpenny*³, *P. Colman*³. 1) Clinical Pharmacogenomics, Pfizer Global Research and Development (PGRD), Sandwich Laboratories, Kent, UK; 2) Discovery Pharmacogenomics, PGRD, Groton Laboratories, CT, USA; 3) Biostatistics and Reporting, PGRD, Sandwich Laboratories, Kent, UK.

The role of sGC in the NO-signalling pathway and the regulation of blood pressure together with the observation of altered sGC subunit expression in rat models suggest that they are good candidate genes for hypertension. A total of 24 (single nucleotide polymorphisms) SNPs in the four sGC subunit genes (6 each in GUCY1A3 and GUCY1B3, 5 in GUCY1A2 and 7 in GUCY1B3 gene were genotyped in 371 hypertensive and 360 control subjects of black South African origin.

A statistically significant association was observed between one SNP (A+6312G) present in intron 2 of the GUCY1A2 gene and hypertension. The estimated increased risk of hypertension in the total population, adjusting for the effects of age, gender and BMI ranged from 1.9 (1.38-2.61) for possession of one additional rare G allele to 3.6 (2-6.81) for two additional rare alleles ($p=0.001$). No other SNP tested in GUCY1A2 or any other sGC gene was associated with disease. This is the first study to identify a genetic association between an sGC subunit gene and hypertension in humans. It is unclear how the GUCY1A2 intron 2 SNP predisposes to hypertension, it may occur in a regulatory region of the gene or may influence splicing, alternatively the association may be due to linkage disequilibrium with SNPs in the promoter of the gene that have yet to be studied. A study to replicate these findings is currently underway in a second black population.

Polymorphisms in antioxidative defences. The Generale Study. A. Rautanen¹, G.A. Fowkes², U. Diczfalusy³, J. Kaikkonen⁴, J. Kere¹, A. Lee², J. Luedemann⁵, K. Nyysönen⁴, H.E. Poulsen⁶, P. Prati⁷, R. Riemersma², F. Rodeghiero⁸, E. Stensland-Bugge⁹, D. Stirling², A. Tosetto⁸, T.-P. Tuomainen⁴, J.T. Salonen⁴. 1) Finnish Genome Center, and Department of Medical Genetics, University of Helsinki, Finland; 2) Wolfson Unit for Prevention of Peripheral Vascular diseases, and Cardiovascular Research Unit, University of Edinburgh, UK; 3) Department of Medical Laboratory Sciences and Technology, Huddinge University hospital, Huddinge, Sweden; 4) Research Institute of Public Health, University of Kuopio, Finland; 5) Institute of Epidemiology and Social Medicine, University of Greifswald, Germany; 6) Department of Clinical Pharmacology, Rigshospitalet, Copenhagen, Denmark; 7) Department of Neurology, Gervasutta Hospital, Udine, Italy; 8) Department of Hematology, San Bortolo Hospital, Vicenza, Italy; 9) Institute of Community Medicine, University of Tromsø, Norway.

The GENERALE is a European multi-centre prospective follow-up study on genetic and environmental risk factors for ageing-related atherosclerosis and cardiovascular events. A subset of IMT, AMI, and stroke cases and matched controls were chosen from five large European population cohorts. The primary hypothesis is that oxidative stress with certain genetic backgrounds enhances atherosclerosis. In the current sub-study we measured the association between candidate gene polymorphisms involved in antioxidative defenses (glutathione peroxidase (GPX1), glutathione S-transferases, haptoglobin, paraoxonases (PON), superoxide dismutase (SOD), myeloperoxidase) and antioxidant enzyme activities in addition to F2-isoprostanes. Polymorphisms in GPX1, PON1, and PON2 genes were significantly associated with the enzyme activities, unlike SOD2 polymorphism. F2-isoprostanes, biomarkers of lipid peroxidation, were correlated with several tested polymorphisms. Interaction of background variables (e.g. smoking) was taken into account in the multivariate model. The allele frequencies of candidate polymorphisms varied between different parts of Europe, the prevalence of GPX1-Pro197Leu (48% in Finland, 29% in Italy), and GSTP1-Ile104Val (25% in Finland, 36% in Germany) differing most among Europeans.

Association between TAFI genotype and risk of MI in white Caucasian male subjects selected from individuals enrolled in a cardiovascular clinical trial. *J. Richmond*¹, *P. Milos*¹, *A. Power*², *S. Bujac*³, *M. Penny*³. 1) Discovery Pharmacogenomics, Pfizer Global R&D, Groton, CT, USA; 2) Clinical Pharmacogenomics, Pfizer Global R&D, New London, CT, USA; 3) Clinical Pharmacogenomics, Pfizer Global R&D, Sandwich, Kent, UK.

Thrombin-activatable fibrinolysis inhibitor (TAFI) has a role in the regulation of fibrinolysis and has been implicated as a risk factor in cardiovascular disease (CVD). The TAFI gene is polymorphic and there is evidence that plasma TAFI antigen levels are genetically controlled. Published data on TAFI genotype and risk of CVD are conflicting. Data suggest that genetic variants that correlate with low levels of TAFI antigen are associated with a decreased risk of DVT and increased risk of MI.

Forty-eight healthy volunteers with TAFI antigen measurements were genotyped for 7 single nucleotide polymorphisms (SNPs) in the TAFI gene. Three groups of subjects were defined from a population of white Caucasians enrolled in the Atorvastatin Comparative Cholesterol Efficacy and Safety Study (ACCESS): 1- Subjects with an historical MI- (340 males, 135 females); 2- Subjects without an historical MI and with atherosclerosis (573 males, 598 females); 3- Subjects without a historical MI and no evidence of atherosclerosis (310 males, 397 females). Multivariate association between TAFI genotype and MI status was carried out as well as logistic regression with adjustment for any confounders. Two TAFI SNPs accounted for approximately 50% of the variability in TAFI antigen in healthy volunteers. A significant association between low TAFI antigen predicting genotype and increased risk of MI was observed in males in the ACCESS study. The estimated increased risk of MI ranged from 1.2 in men possessing one low TAFI antigen predicting allele up to 2.1 for two alleles. There was also evidence that the effect of TAFI genotype on risk of MI is not the same in males and females. Establishing the relationship between TAFI genotype, antigen level, and activity is the next step in understanding the association between TAFI and CVD.

Association study of the OLR1 gene with coronary artery disease and acute myocardial infarction. *E. Trabetti¹, M. Biscuola¹, U. Cavallari¹, G. Malerba¹, R. Galavotti¹, S. Friso², D. Girelli², N. Martinelli², O. Olivieri², F. Pizzolo², R. Corrocher², P.F. Pignatti¹.* 1) Dept.Mother-Child & Biol-Genetics, Univ Verona, Verona, Italy; 2) Dept. Clinical & Experimental Medicine, Univ. Verona, Verona, Italy.

Oxidised LDL receptor 1 (OLR1 or LOX 1) has been considered a good candidate gene for atherosclerosis and myocardial infarction since it is upregulated by various proatherogenic stimuli and induced in vitro by inflammatory cytokines. Seven polymorphisms have been identified in the OLR1 gene and associations between these SNPs and coronary artery disease (CAD) or acute myocardial infarction (AMI) have been reported in different populations. Aim of our study was to investigate an association of OLR1 gene polymorphisms with AMI and CAD in a North East Italian population sample. For this purpose, a total of 575 individuals subjected to coronarography were genotyped for the following three OLR1 gene SNPs: IVS4-73CT, IVS4-14AG and 501GC. Allele and genotype frequencies were determined in the following four groups of patients: 165 AMI, 156 non AMI, 294 CAD, 281 non CAD. The distribution of genotypes in each group was in Hardy-Weinberg equilibrium. We confirmed a complete L.D. between the two IVS4 SNPs. No statistically significant difference was observed in allele or genotype distribution in AMI or CAD patients compared to controls. In conclusion, the data here reported do not provide evidence for strong association of AMI or CAD with the OLR1 gene. Further studies on OLR1 and on OLR1/other gene polymorphisms interaction could probably lead to explain the genetic involvement in AMI and CAD in the Italian population.

Association of a SNP in MGEA5 encoding O-GlcNAcase with diabetes in Mexican Americans. *D. Lehman¹, D.-J. Fu², K. Hunt¹, R.J. Leach¹, R. Arya¹, H. Abboud¹, R. Duggirala³, T. Dyer³, H.H.H. Goring³, J. Blangero³, M.P. Stern¹.*
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Excess O-glycosylation of proteins by O-linked N-acetylglucosamine (O-GlcNAc) may be involved in the pathogenesis of type 2 diabetes. O-GlcNAcase, encoded by MGEA5 on 10q24.1-q24.3, reverses this modification by catalyzing the removal of O-GlcNAc. STZ, a beta cell toxin used to induce diabetes in rodents, acts by inhibiting O-GlcNAcase. We have previously reported linkage of type 2 diabetes and age at diabetes onset to an overlapping region on chromosome 10q in the San Antonio Family Diabetes Study (SAFADS). We, therefore, investigated MGEA5 as a positional candidate in this population of Mexican Americans. To identify variants, all exons and flanking intronic sequence, 5 and 3 UTRs, 1 kb upstream of exon 1, and all of introns 10 and 11 were resequenced in 22 diabetic and 22 non-diabetic subjects. We identified 24 SNPs of which 19 are novel (minor allele freq is 0.02 to 0.25). All SNPs were genotyped in 436 individuals from 27 families whose data were used in the original linkage report. Each SNP was tested for association with the traits diabetes and diabetes age-of-onset using a measured genotype approach, as implemented in SOLAR. Significant association of nonredundant SNP LLY-MGEA5-14 (mg14, minor allele freq is 0.04), located in intron 10, was observed with the traits diabetes age-of-onset ($p=0.001$) and diabetes ($p=0.012$). We investigated whether this variant was responsible for the original linkage signal by including it as a fixed effect in the linkage analysis, leading to a drop in LOD score from 3.77 to 2.84. These results suggest that this variant may partially account for diabetes risk in Mexican Americans. SNP mg14 is located within intron 10, which contains an alternate stop codon, so this variant could alter expression of MGEA5 isoforms, although functional studies will be necessary to confirm this. Alternatively the mg14 variant may be in LD with true functional variants in this region.

Single nucleotide polymorphisms in GRPR gene in male patients with autism. *L. Zhang¹, R.J. Schroe², Y. Jiang¹, C. Obie¹, D. Valle^{1,3}, R. Stevenson², T. Wang¹.* 1) Dept Human Genetics, JHMI, IGM, Baltimore, MD; 2) Greenwood Genetic Center, SC; 3) Howard Hughes Medical Institutions.

Autism is a neuropsychiatric disorder characterized by severe social and communication deficits with restricted or repetitive behaviors or interests. Genetic factors play important roles in the development of autism and males are four times more likely to be affected than females. To identify X-linked autism susceptibility genes, we studied X-linked gene expression in lymphoblast cell lines from males with autism using a combination of X chromosome cDNA microarray, quantitative real-time PCR, and Northern blot analysis. We found reduced expression of gastrin-releasing peptide receptor (GRPR) gene in several cell lines. In follow up sequence analysis of GRPR in 92 probands, we identified 13 SNPs: 5 synonymous cSNPs, 3 of which not previously reported; and 8 were found in introns or 5- and 3-UTRs. Interestingly, we identified a single G>A substitution in the 5-UTR in 9 of 92 (10%) probands and in 5 of 242 (2%) controls ($X^2=9.88$, $p<=0.01$). In all tested cell lines (n=4) carrying the G>A substitution steady state level of GRPR mRNA were reduced to less than 20% of normal. GRPR is a G-protein coupled receptor that is expressed in the limbic system, an area of the brain involves in emotion, affection and attention. Interestingly, a previously reported female with autism had an X;8 translocation with the breakpoint in intron-1 of GRPR (Ishikawa-Brush et al., 1997), and a recent study of Grpr knockout mouse revealed an enhanced amygdala-mediated long-term memory for fear. These observations suggest that further evaluation of the genetics and function of GRPR are warranted to understand its possible roles in autism.

Mutations in the Genes for MODY are not a Common Cause of Early-Onset Type 2 Diabetes in Minority

Populations. *H. Allayee*¹, *D. Parker*², *A. Stammreich*², *J. Canales*², *G. Scheel*², *A. Lusic*³, *E. Ipp*². 1) Dept Prev Med, Univ Southern Calif, Los Angeles, CA; 2) Dept of Med, Harbor-UCLA Med Cntr, Torrance, CA; 3) Dept of Med, UCLA, Los Angeles, CA.

In recent years, type 2 diabetes is increasingly diagnosed at younger ages, particularly in minority populations such as Mexican-Americans and African-Americans. Maturity onset diabetes of the young (MODY) is defined classically as a Mendelian dominant form of type 2-like diabetes developing before age 25 in a proband or at least one family member. To determine whether mutations in the genes for the most common causes of MODY could account for the early-onset type 2 diabetes observed in minority patients, we screened the coding regions and flanking intronic boundaries of the hepatocyte nuclear factor 1-alpha (HNF1a), HNF4a and glucokinase genes by heteroduplex analysis and direct sequencing. The study population (n=29) consisted of 17 Mexican-Americans and 9 African-Americans families that fulfilled the traditional diagnostic criteria for MODY. Apart from previously described common polymorphisms, no mutations were found in 28 of the probands. One African-American subject, who developed type 2 diabetes at the age of 25, was heterozygous for a Tyr322Cys substitution in exon 5 of HNF1a. The sibling and father of the proband developed diabetes at age 26 and 52, respectively, and were also heterozygous carriers of the variant. Physiological testing of these family members revealed a significant insulin secretion defect in response to a bolus glucose infusion. Using the PolyPhen program, which predicts the functional consequence of amino acid substitutions, a cysteine residue at position 322 would severely damage the function of HNF1a. Taken together, these results suggest that the Y322C substitution is most likely a previously undescribed disease-causing MODY mutation. Furthermore, the very low frequency of mutations in the common MODY genes in a population enriched for this disorder (i.e., fulfill the classical definition for MODY), suggests that diagnostic criteria for this early-onset form of diabetes should be carefully re-evaluated, particularly in those patients of minority descent.

Variation in *NCB5OR*: studies of relationships to type 2 diabetes, MODY, and gestational diabetes mellitus. G. Andersen¹, L. Wegner¹, C.S. Rose¹, J. Xie², H. Zhu², K. Larade², A. Johansen¹, J. Ek¹, J. Lauenborg³, T. Drivsholm⁴, K. Borch-Johnsen^{1,4}, P. Damm³, T. Hansen¹, H.F. Bunn², O. Pedersen^{1,5}. 1) Steno Diabetes Center, Gentofte, Denmark; 2) Brigham and Womens Hospital, Harvard Medical School, Boston, USA; 3) Obstetric Clinic, National University Hospital, Rigshospitalet, Copenhagen, Denmark; 4) Research Centre for Prevention and Health, Glostrup University Hospital, Glostrup, Denmark; 5) Faculty of Health Science, Aarhus University, Aarhus, Denmark.

Recent data show that homozygous *Ncb5or*^{-/-} knock-out mice present with an early-onset non-autoimmune diabetes phenotype. Furthermore, genome-wide scans have reported linkage to the chromosome 6q14.2 region close to the human *NCB5OR*. We therefore considered *NCB5OR* to be a biological and positional candidate gene and examined the coding region of *NCB5OR* in 120 type 2 diabetic patients and 63 patients with maturity-onset diabetes of the young (MODY) using denaturing high performance liquid chromatography (dHPLC). We identified a total of 22 novel nucleotide variants. Three variants (IVS5+7del(CT), Gln187Arg, and His223Arg) were genotyped in a case-control design comprising 1246 subjects (717 type 2 diabetic patients and 529 subjects with normal glucose tolerance). The Gln187Arg variant was also genotyped in 140 women with previous gestational diabetes mellitus (GDM). In addition, four rare variants were investigated for co-segregation with diabetes in multiplex MODY or early-onset type 2 diabetes families. The IVS5+7del(CT) variant was associated with common late-onset type 2 diabetes ($p = 0.03$); however, we failed to relate this variant to any diabetes-related quantitative traits among the 529 control subjects. There was no evidence for co-segregation of mutation genotype with disease affection status among families with MODY or early-onset type 2 diabetes. Thus, variation in the coding region of *NCB5OR* is not a major contributor in the pathogenesis of non-autoimmune diabetes.

Polymorphisms in the FOXP3 gene confer susceptibility to Type 1 Diabetes. *M. Bjoernvold¹, L.C. Stene², G. Joner³, K. Dahl-Joergensen³, K.S. Roenningen², B. Skinningsrud¹, D.E. Undlien¹.* 1) Medical Genetics, University of Oslo, Norway; 2) Norwegian Institute of Public Health; 3) Department of Pediatrics, Ulleval University Hospital.

Type 1 Diabetes (T1D) is a T-cell mediated autoimmune disease which leads to destruction of insulin producing beta-cells in the pancreas. It is a genetically complex disease. The FOXP3 gene located on chromosome Xp11.23 plays an important role in normal immune homeostasis. It encodes a DNA binding protein which is a transcription-repressor protein involved in regulatory T cell activity. It is expressed in CD4+CD25+ regulatory T-cells, and mutations in this gene is known to cause a rare, serious disease (IPEX, OMIM #304790) characterized by severe generalized autoimmunity. Two independent studies have analyzed common polymorphisms in FOXP3 in T1D with conflicting results. A Japanese study found that a polymorphism in the promoter region were associated with T1D while a study from Sardinia found no evidence for association. We have genotyped two microsatellite polymorphisms in intron zero and 5, respectively in 360 T1D children and their parents and analyzed for association with T1D using the transmission/disequilibrium test (TDT). The markers were in strong linkage disequilibrium. Our result did indicate an association between FOXP3 and T1D. The (TC)₂₆ allele of the dinucleotide repeat in intron 5 was transmitted 76.9% of times compared to the expected 50% ($p = 0.005$). Our results did also suggest an interaction between FOXP3 and HLA class II susceptibility alleles. Our results differ from those observed among Japanese. In the Japanese population an association with the microsatellite in intron zero was found. This could imply that there is an other hitherto unidentified polymorphism in the region that is primarily involved, and that the linkage disequilibrium pattern in Norway and Japan differ, leading to association with different markers in the two populations. We are now performing an independent case control study with about 500 cases and 1700 controls to see if we can replicate these results.

SNP fine mapping on chromosome 14q23-q24 for type 2 diabetes in Finns. *L.L. Bonnycastle¹, A.U. Jackson², P. Chines¹, N. Riebow¹, L.J. Scott², K.L. Mohlke¹, N. Narisu¹, W.L. Duren², H. Zhuang¹, M. Li², M.R. Erdos¹, T.T. Valle³, K. Silander³, J. Tuomilehto³, R.N. Bergman⁴, M. Boehnke², F.S. Collins¹.* 1) NHGRI, Bethesda, MD; 2) U. Michigan, Ann Arbor, MI; 3) Nat'l Public Health Inst., Helsinki, Finland; 4) U. Southern California, Los Angeles, CA.

The aim of the Finland-United States Investigation of NIDDM Genetics (FUSION) study is to identify genes that predispose to Type 2 Diabetes (T2D) and contribute to diabetes-related quantitative traits. A genome scan of 737 Finnish affected sibling pair families with microsatellite (STRP) fine-mapping to a density of 1.4 cM, yielded a maximum LOD score (MLS) of 2.26 at 55.5 cM on chromosome 14q23-q24. This MLS is the third largest in the 737 families. Ordered subset analysis of families with low resting pulse and low triglycerides generated LOD scores 4.97 ($p=.0049$) at 55.0 cM, and 4.96 ($p=.0061$) at 61.0 cM, respectively. A QTL for HDL-to-cholesterol ratio (MLS=1.88) among affected individuals was observed at 49.5 cM. When conditioning on families with negative linkage evidence on chromosome 6 at 77 cM, the T2D linkage peak increased to MLS=5.52. We are currently engaged in extensive fine mapping of a 23 cM (20 Mb) region, centered on the linkage peak and including our 1-LOD support interval. We selected a total of 1381 SNPs from HapMap SNPs (www.hapmap.org), selecting SNPs with $r^2 < 0.8$, non-synonymous dbSNP cSNPs, and additional dbSNPs to fill large gaps (>30kb) and gaps with low linkage disequilibrium between the HapMap SNPs. The resulting average SNP density is one per 13 kb. These SNPs are being genotyped using the Illumina platform on 806 probands from T2D families, 605 affected and unaffected siblings of these probands, and 657 normoglucose tolerant controls. Results to be reported will include 1) comparison of linkage results from SNPs and STRPs; 2) association analyses of SNPs and haplotypes with disease status and disease-related quantitative traits; and 3) an assessment of whether the association evidence can explain the linkage signals.

Follow-up of a Type 2 Diabetes Whole-Genome Linkage Study. *L.P. Briley¹, A.P. Philip², M.C. Karnoub², M.G. Ehm², P.L. St.Jean², R.G. Winkler³, G. Chandra^{4,5}, D.P. Yarnall¹, A.J. Nelsen², D.D. Kelly², J.H. Charnecki², K.W. Johansson², M.J. Wagner^{4,6}.* 1) Portfolio Genetics, GlaxoSmithKline, RTP, NC; 2) Genetic Data Sciences, GlaxoSmithKline, RTP, NC; 3) Bioinformatics, GlaxoSmithKline, RTP, NC; 4) Genetics Research, GlaxoSmithKline, RTP, NC; 5) United States Patent and Trademark Office, Washington, D.C. (present affiliation); 6) First Genetic Trust, Deerfield, IL. (present affiliation).

Type 2 diabetes is a disease with both genetic and environmental components that causes many long-term healthcare complications and costs billions of dollars annually. GlaxoSmithKline undertook a series of genetic studies to follow up the genome-wide type 2 diabetes linkage study reported in 2000. Fine-mapping of SNPs was performed in three of the linkage regions identified by the whole-genome scans. Association was seen in these regions for a number of SNPs using a variety of analysis methods. Recently, a large replication study in four additional populations was completed on 251 SNPs from associations in the fine-mapping studies, as well as candidate genes. For comparison, we also studied several SNPs in the known diabetes susceptibility gene PPAR-gamma. In the chromosome 1q linkage region (18 MB), two SNPs 24 KB apart showed association in multiple tests in at least three populations. In the chromosome 12q linkage region (14 MB), one SNP showed association in at least three populations in multiple tests. In the chromosome Xq linkage region (18 MB), five SNPs in five different locations throughout the region showed association in multiple populations with multiple tests. This work may lead to increased understanding of type 2 diabetes and to the identification of novel susceptibility genes and drug target pathways.

Examination of PPP1R3B as a candidate gene for the type 2 diabetes and MODY loci at 8p23. *J.S. Dunn¹, W.M. Mlynarski^{1,2}, M.G. Pezolesi¹, C. Powers¹, A.S. Krolewski^{1,2}, A. Doria^{1,2}.* 1) Genetics and Epidemiology, Joslin Diabetes Center, Boston, MA; 2) Department of Medicine, Harvard Medical School, Boston, MA.

The product of the *PPP1R3B* gene (G_{T}) is the regulatory subunit of a phosphatase involved in the modulation of glycogen synthesis in the liver and skeletal muscle. The *PPP1R3B* gene is located on chromosome 8p23 in a region that is linked with T2D and maturity-onset diabetes of the young (MODY) in the Joslin Study on the Genetics of Type 2 Diabetes (Pezolesi et al., 2004, Kim et al., 2004). We examined whether sequence variants at the *PPP1R3B* locus are responsible for the linkage with diabetes observed at this location. The two exons of this gene and 1.5 kb of the 5' flanking region were sequenced in the probands of 13 Joslin families (8 with type 2 diabetes, 5 with MODY) showing the strongest evidence of linkage at 8p23. We observed a total of 20 SNPs: two in the 5' flanking region, one in the intron (9 bp 5' of exon 2), and 17 in the 3' UTR. Four of these SNPs, representative of all others, were then genotyped in 79 type 2 and 21 MODY probands from the Joslin Study and in 91 unrelated controls. No statistically significant association with type 2 diabetes or MODY was observed. Results were similar when the analysis was restricted to families having the strongest evidence of linkage. Additionally, we examined the gene for the occurrence of splice variants. By means of RT-PCR, we identified an alternative transcript in which 8 bp are added at the 5' end of exon 2. Further study of this polymorphism revealed that expression of this larger transcript was contingent on the presence of the minor allele of an intronic SNP creating a new splice acceptor site. However, no association of type 2 diabetes or MODY was observed for this variant. In summary, variability in *PPP1R3B* does not appear to contribute to diabetes in our mostly Caucasian families. However, a role cannot be excluded in other populations such as Japanese, among whom linkage to diabetes is also observed at 8p23 and a non-synonymous mutation has been detected in the *PPP1R3B* gene.

Thrifty genotype effect on type 2 diabetes mellitus, in a sample in North West Colombia. *C. Duque¹, F. Uribe², G. Latorre², A. Villegas², L. Franco¹, N. Pineda^{1, 3}, G. Bedoya¹, A. Ruiz-Linares^{1, 3}.* 1) Laboratorio de Genética Molecular, Universidad de Antioquia. Medellín, Colombia; 2) Departamento de Medicina Interna, Facultad de Medicina, Universidad de Antioquia. Medellín, Colombia; 3) Galton Laboratory, Department of Biology, University College London, U.K.

One of the causes of type 2 diabetes mellitus (T2DM) could be obesity, since; it is strongly associated to insulin resistance, the main sign of T2DM. It has been postulated that the obesity susceptibility is caused by the interaction of mitochondrial (mtDNA) and nuclear variants, denominated Thrifty Genotype (TG), which have remained in Human migrant populations, as the Amerindian ones. In order to evaluate this interaction, we assessed the Amerindian TG effect on T2DM susceptibility in the population of Antioquia (Colombia) considered as a tri-ethnic admixture. We compared between cases and controls haplogroups and haplotypes frequencies in mtDNA as well as, the values of ancestry indexes obtained by seven nuclear markers. Seventy five percent of the mitochondrial haplogroups were of Amerindian origin and no differences in their frequencies were found. The values of haplotype variability estimators were significantly high in controls than in cases ($0.0004 < P_s < 0.006$). Differences were detected between the two groups in linkage disequilibrium and pairwise difference distributions. Four nucleotidic substitutions were found associated to the controls and one to the cases. The haplotypic variability in nuclear loci ($p = 0.009$) as well as the European admixture grade ($p = 0.016$) were higher in controls than in the cases. This is in concordance with the average values of Amerindian and African ancestry indexes that were higher in the cases (AAI $p = 0.0004$ and AfAI $p < 0.0001$ respectively). We found a direct relation between high values of AAI and the frequencies of Amerindian haplogroups and haplotypes. In this study we found for the first time, an evidence about of effect of TG determined by the interaction between high values of AAI, AfAI and mtDNA polymorphisms, on T2DM susceptibility in the population of Antioquia, also such effect would be modulated by the genetic variability in nuclear and mitochondrial genomes.

Large-scale genetic epidemiological and functional studies of the Thr130Ile and Val255Met polymorphisms in the Hepatocyte Nuclear Factor-4alpha gene in relation to type 2 diabetes. *J. Ek¹, C.S. Rose¹, D.P. Jensen¹, C.*

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HNF4A encodes an orphan nuclear receptor that plays crucial roles in regulating hepatic gluconeogenesis and beta-cell function. *HNF4A* is located on chromosome 20q13 in a region which in several studies has shown consistent linkage with type 2 diabetes mellitus (T2D), and recently, several common polymorphisms near the gene were shown to associate with T2D. Furthermore, rare mutations in the gene cause MODY1. The aim of the present study was to examine two rare missense polymorphisms in *HNF4A*, Thr130Ile and Val255Met, for altered function of HNF4A and for association with T2D. We have examined these polymorphisms by; 1) *in vitro* in transactivation studies using a *HNF4A* insert in an pcDNA3 vector and an *HNF1A* promoter driven dual *Renilla* and Firefly Luciferase reporter plasmid, and 2) by genotyping the variants in 1409 T2D patients and in 4726 glucose tolerant Danish white subjects. Both the Thr130Ile (76%, $p=0.04$) and the Val255Met (73%, $p=0.02$) variants showed significant decrease in transactivation level compared with wildtype. Further, the Thr130Ile variant had a significant increased allele frequency among T2D patients (4.2% (95% CI: 3.4-4.9)) compared to glucose tolerant subjects (3.4% (3.1-3.9)) (OR=1.26, $p=0.04$). The rare Val255Met polymorphism had a similar frequency among type 2 diabetic patients (0.1% (0-0.2)) and in glucose tolerant subjects (0.1% (0-0.2)). In conclusion, the Thr130Ile and the Val255Met polymorphisms decrease the transactivation activity of *HNF4A* and the Thr130Ile polymorphism increases risk of diabetes in the Danish Caucasian population.

Association of the Estrogen Receptor 1 gene with Type 2 Diabetes in African American and Caucasian American populations. *C.J. Gallagher^{1,4}, C.D. Langefeld², J.C. Mychaleckyj^{3,4}, J.N. Hirschhorn⁵, B. Henderson⁶, C.J. Gordon⁴, B.I. Freedman³, S.S. Rich², D.W. Bowden^{1,3,4}, M.M. Sale^{3,4}.* 1) Dept Biochemistry, Wake Forest Univ Sch Medicine, Winston-Salem, NC; 2) Dept Public Health Sciences, Wake Forest Univ Sch Medicine, Winston-Salem, NC; 3) Dept Internal Medicine, Wake Forest Univ Sch Medicine, Winston-Salem, NC; 4) Dept Human Genomics, Wake Forest Univ Sch Medicine, Winston-Salem, NC; 5) Departments of Genetics and Pediatrics, Harvard Medical School and Program in Medical and Population Genetics, Broad Institute, MIT/Harvard, Boston, MA; 6) Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA.

We have completed a genome-wide scan for type 2 diabetes mellitus (T2DM) in 638 African American (AA) affected sibling pairs and found evidence for linkage (LOD=2.26) at 6q24-q27. The estrogen receptor alpha gene, ESR1, is in the linkage region. Mice and humans lacking a functional copy of ESR1 exhibit impaired glucose tolerance and obesity. We genotyped 53 SNPs that tag common haplotypes over 322 kb of the ESR1 gene in 380 AA T2DM cases and 276 AA controls. We performed tests of association on single SNPs and haplotypes reconstructed using a standard EM-algorithm. Two SNPs (10.9 kb separation) in intron 2 were associated with T2DM ($P=0.000037$ and 0.0131); common haplotypes within a haplotype block spanning intron 1 - intron 2 were also associated (global $P=0.00004$, likelihood-based permutation test). Additional SNPs were genotyped across 41 kb of intron 1-intron 2. 17 SNPs form 3 contiguous haplotype blocks in AA, and common haplotypes within each block were associated with T2DM (global $P<0.0001$). These 17 SNPs were also genotyped in 300 European American (EA) T2DM cases and 310 EA controls. There were two significantly associated single SNPs ($P=0.0150$ and $P=0.0192$) and an associated 4-SNP haplotype ($P=0.033$) in the EA population. We present the first report of a T2DM gene positionally identified in an AA population. While these results need to be replicated in additional populations, they suggest that common variants in the intron 1 - intron 2 region of ESR1 contribute to T2DM susceptibility.

Metanalysis of the Insulin Promoter Factor-1 (Ipf-1) D76N Missense Mutation in an European Type 2 Diabetes Mellitus (T2D) Population. *J.F. Habener^{1,2,3}, E. Milord⁴, C. Gragnoli^{1,2,5}*. 1) Mol Endocrinology, MGH, Boston, MA; 2) HMS, Boston, MA; 3) HHMI, Boston, MA; 4) Millennium Ph, Inc, Cambridge, MA; 5) Bios Health Center, Rome, Italy.

Ipf-1 is the MODY4 gene, transcription factor regulator of pancreas development and of expression of beta-cell specific genes. A common missense mutation is Ipf-1 D76N, which has been shown to increase risk for T2D, to inhibit insulin promoter activity, and to decrease glucose-stimulated insulin-secretion in healthy subjects. As Ipf-1 D76N seems to be mostly a variation of European origin, we calculated the risk for T2D in D76N carriers by performing a metanalysis of Ipf-1 published data in all European populations including our Italian T2D cohort, which consists of 90 late-onset and 40 early-onset T2D subjects and 50 controls (Gragnoli C, 1998 and 2003). We retrieved all data on D76N in different population (French/UK/Danish-Italian/Swedish) including subjects with late-onset T2D, early-onset T2D and MODY and tested D76N for HWE and for association with T2D by Relrisk using the total cases, and subsets with late-onset or early-onset T2D (including MODY). The same tests were performed separately in the Italian cohort, and separately in the late-onset (early-onset is negative). D76N frequency in Italian late-onset T2D cohort is 0.01 and in total early-onset and late-onset Italian T2D cohort is 0.007. D76N frequency in 247 European early-onset T2D patients is 0.012, in 887 European late-onset T2D patients is 0.015, in 1134 total European T2D patients is 0.014, and in 1048 European controls is 0.007. D76N is in complete HWE in Italian, European and control groups. The RR for D76N in Italian late-onset T2D is 1.69 (OR=0.15-19.13, P=0.45), in Italian early- and late-onset T2D is 1.17 (OR=0.10-13.19, P=0.53), in European early-onset T2D cohort is 1.60 (OR=0.42-6.07, P=0.48), in European late-onset T2D cohort is 2.08 (OR=0.87-4.99, P=0.09) and in European early- and late-onset T2D cohort is 1.98 (OD=0.85-4.60, P=0.10). D76N does not achieve statistical significance in the association with T2D in the European cohort tested, however there is a trend towards association mainly with late-onset T2D.

Haplotype construction of the FRDA gene and evaluation of its role in Type 2 Diabetes. *J. Holmkvist¹, P. Almgren¹, H. Parikh¹, M. Zucchelli², J. Kere², L. Groop¹, C.M. Lindgren².* 1) Dept. of Endocrinology, Malmo University Hospital, S-205 02 Malmo, Sweden; 2) Dept. of Biosciences at Novum, Clinical Research Center, Huddinge University Hospital, 141 86 Stockholm, Sweden.

Friedreichs ataxia (FRDA) is a common neurodegenerative disease and studies have shown that FRDA is caused by an expansion of an intronic (GAA)_n repeat in the X25 gene on chromosome 9q13. Almost 20% of Friedreichs ataxia patients develop type 2 diabetes (T2D), and an additional 10% develop milder disturbances of glucose tolerance. Several groups have investigated the role of the X25 repeat in T2D. Others and we have detected linkage in T2D families to chromosome 9p13-q21. First, we investigated the trinucleotide repeat allele distribution in a sample of 220 transmission disequilibrium trios with IFG (n=50), IGT (n=72) and T2D (n=98) from the Botnia study. However, we failed to detect any association or transmission distortion to disturbed glucose tolerance. We performed a meta analysis to evaluate whether our data was consistent with previous findings and found significant evidence against association between the X25 intermediate trinucleotide repeat alleles and disturbed glucose tolerance (OR=1.04, 95%CI [0.86-1.26], p=0.7). This suggests that the repeat itself does not influence the development of type 2 diabetes. To test whether there was excess transmission of any of the present variants and/or haplotypes we examined linkage disequilibrium and haplotype structure over the X25 gene using 18 single nucleotide polymorphisms covering 67 kb. One SNP, rs2498429, 8 kb downstream the X25 gene was nominally associated with T2D in the trios (p=0.02) and (p=0.08) in a follow-up case control study (n=529/329). The combined analysis of the case-control and trio data supports the initial association (OR=1.66 95%CI [1.13-2.44], p<0.01). In addition, haplotype transmission disequilibrium tests (TDT4) showed an over transmission of haplotypes containing the rare variant of rs2498429. In conclusion, our data do not support a role for the Friedreichs ataxia gene in the pathogenesis of T2D, but points to the possibility that genetic variation downstream the X25 gene on chromosome 9 could increase risk for T2D.

Polymorphisms of Candidate Genes for Type 2 Diabetes in Asian Indian Males from central Trinidad. *HA. Keenan*^{1,2}, *CH. Bunker*², *MM. Barmada*², *RE. Ferrell*², *NI. Petro*², *A. Doria*¹, *DE. Kelley*². 1) Dept Genetics & Epidemiology, Joslin Diabetes Ctr, Boston, MA; 2) University of Pittsburgh, Pittsburgh, PA.

Diabetes is a complex disease with an established genetic component. We previously documented a type 2 DM prevalence rate of 34% among physically active Asian Indian males, aged 19-65 years, living in central Trinidad. A positive family history was reported by 81%. We examined the frequency of candidate gene polymorphisms in apM1 at position 276, GLUT-4 at codon 383, IRS-1 at codon 972, the pro12ala variation of PPAR-2, and the non-synonymous mutation of SPINK1 gene at codon 117. Among 274 Asian Indian males studied, the T allele of the apM1 gene was less prevalent in those with type 2 DM (18.2% v. 24.6% (p=0.09) than those without the disease, although this was not significant. However, when age adjusted, the genotype TT was significantly protective against type 2 DM in reference to GG, OR:0.15 (95% CI:0.03- 0.73). There was a trend of lower fasting plasma glucose levels among those who were homozygous for the T allele (101.9 mg/dL compared with 120.7 mg/dL (GG) and 123.2 mg/dL (GT), p= 0.29). Adiponectin levels in a subset (n=48) of individuals matched for BMI and age were not significantly associated with the apM1 genotype (GG 7.8 +/-6.2 ng/mL, GT 8.2 +/- 3.5 ng/mL, TT 7.7 +/-3.2 ng/mL, p=0.9). A borderline protective association of type 2 DM and the glycine to arginine mutation of codon 972 of IRS-1 was found (p=0.07). BMI was lower among those carrying the mutation (24.9 kg/m² v. 26.4 kg/m², p=0.06). Other than BMI, there were no significant differences in measures of fasting plasma glucose, insulin, HDL, LDLc, triglycerides, or total cholesterol between the genotypes of IRS-1. No associations were found between type 2 DM and polymorphisms at the other three loci. However, we document a higher proportion of homozygotes for the variant allele of the PPAR- 2 gene than in the Caucasian population (~3%). This preliminary study of polymorphisms in a population of Asian Indians complements the sparse data previously available regarding the genetic epidemiology of type 2 DM in this ethnic group and demonstrates an association of SNP 276 of apM1 with type 2 DM in Asian Indians.

Interleukin-18 promoter polymorphisms are not associated with type 1 diabetes in a UK population. R.J.L.

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Interleukin-18 is a proinflammatory cytokine whose levels are increased in the subclinical stage of type 1 diabetes. Two single nucleotide polymorphisms at positions -607(C>A) and -137(G>A) of the IL-18 promoter have been reported to cause differences in transcription factor binding. The H4TF-1 nuclear factor binding site is altered at position -137, while a potential cAMP-responsive element-binding protein binding site is disrupted at position -607. Previous studies in Polish and Japanese populations have found an association between IL-18 promoter polymorphisms and type 1 diabetes. Association between IL-18 -607C/-137G and CTLA-4GG at nucleotide position 49 in exon 1 has also suggested a possible gene-gene interaction.

We performed case-control and family based association studies by pyrosequencing to assess if the IL-18 promoter polymorphisms were associated with the development of early onset type 1 diabetes in the Northern Ireland population. In addition we examined the relationship between IL-18 and CTLA-4 genotypes.

Chi-squared analysis of genotype and allele frequencies for the IL-18 polymorphisms in cases (n=129) compared to controls (n=426) found no significant difference ($p>0.05$). Assessment of allele transmission distortion in the 283 trios (proband and both parents) found no association between IL-18 polymorphisms and the development of type 1 diabetes. Comparison of IL-18 and CTLA-4 genotypes using logistic regression test for interaction did not exhibit association.

In conclusion, IL-18 promoter polymorphisms are not associated with type 1 diabetes in the Northern Ireland population and no interaction between IL-18 and CTLA-4 genes was found.

Association Studies of Kir6.2 E23K and PPAR P12A in an Italian T2D Cohort. *E. Milord*¹, *J.F. Habener*^{2,3,4}, *C. Gragnoli*^{2,3,5}. 1) Millennium Ph, Inc, Cambridge, MA; 2) Mol Endocrinology, MGH, Boston, MA; 3) HMS, Boston, MA; 4) HHMI, Boston, MA; 5) Bios Health Center, Rome, Italy.

The ATP-sensitive K⁺ channel subunit Kir6.2 is critical in glucose-mediated insulin secretion as a link between glucose metabolism and electrical cell activity. Large association studies and meta-analyses of Kir6.2 E23K have reported association with type 2 diabetes (T2D). The peroxisome proliferator-activated receptor (PPAR) is a member of the nuclear hormone receptor superfamily and is important for adipocyte differentiation and insulin sensitivity. Large meta-analyses have reported decreased risk for PPAR P12A for T2D development. Our aim was to test Kir6.2 E23K and PPAR P12A for association with T2D in an Italian cohort (205 patients, 105 controls from Rome). We amplified genomic DNA of all subjects by PCR with primers for E23K and for P12A. PCR products were controlled for amplification quality by 2% agarose electrophoresis gel, purified by Qiagen kit (Qiagen, Valencia, CA) and sequenced on both strands with fluorescein-labelled markers (3700 ABI PRISM). We tested for HWE cases and control genotypes by HWE software and for association with T2D alleles and genotypes by Relrisk. Genotypes were in HWE. Association results are: at E23K site, genotype KK (cases=0.17, controls=0.11) has RR=1.53 (OR=0.75-3.10, P=0.23), EK (cases=0.43, controls=0.41) has RR=1.09 (OR=0.67-1.77, P=0.72), EE (cases=0.39, controls=0.46) has RR=0.74 (OR=0.46-1.20, P=0.22), allele K has RR=1.31 (OR=0.92-1.88, P=0.13), E has RR=0.76 (OR=0.53-1.09, P=0.13); at P12A site, genotype AA (cases=0.004, controls=0.000) has RR=1.55 (OR=0.14-17.26, P=0.47), AP (cases=0.12, controls=0.12) has RR=0.98 (OR=0.48-2.01, P=0.96), PP (cases=0.87, controls=0.87) has RR=0.97 (OR=0.48-1.98, P=0.93), allele A has RR=1.07 (OR=0.54-2.12, P=0.84), P has RR=0.94 (OR=0.47-1.85, P=0.84). This is the first report of E23K and P12A study in Italians. Our study does not reach statistical significance, however extrapolation of our data suggests that Kir6.2 K23 allele and PPAR A12A genotype confer risk to T2D in Italians (13% due to chance, n.s., respectively). Our study enriches the public genotypes pool for further meta-analyses.

IRS 1 Gene Polymorphism in Turkish Type 2 Diabetes Mellitus Patients. *F.E. Orkunoglu¹, H. Mergen², E.S. Bolu³, M. Ozata⁴.* 1) Dept Medical Genetics, Gulhane Sch Medicine, Ankara,Turkey; 2) Dept Biology, Faculty of Science, Hacettepe University, Ankara,Turkey; 3) Dept Endocrinology and Metabolism, Gulhane Sch of Medicine,Ankara, Turkey; 4) Dept Endocrinology and Metabolism,GATA Haydarpaşa Training Hospital,Acibadem-Istanbul, Turkey.

Insulin receptor substrate-1 (IRS-1) is an endogenous substrate for the insulin receptor tyrosine kinase, which plays an important role in insulin signaling. Mutations in the IRS-1 gene are associated in some populations with Type 2 diabetes. To determine whether variation in the IRS-1 gene contributes to genetic susceptibility to Type 2 diabetes, the coding region of IRS-1 gene was analyzed for variation in 70 T2DM subject and 116 control patients using PCR-RFLP and DNA sequencing method. Three single base changes found (Gly972Arg, Ala512Pro, Ser892Gly). There was no significant association found any of these variants with diabetes except for Gly971Arg. This mutation were relatively more common in with 10/70 diabetic patients and 15/116 non-diabetic controls being heterozygous and 1/70 being and 0/116 non-diabetic controls being homozygous for this variant. As a conclusion, Ala512Pro, Ser892Gly, mutations are rare and Met613Val, Ser1043Tyr and Cys1095Tyr mutations are not found. Gly971Arg is more common in our population but may not be a major determiner to genetic susceptibility to Type 2 diabetes.

The T92A SNP in the Deiodinase 2 (DIO2) gene is associated with a reduced risk of type 2 diabetes: evidence for interaction with the PPAR2 gene. *I. Torrente¹, M. Fiorito¹, S. De Cosmo⁴, V. Guida^{1,2}, A. Colosimo^{1,6}, S. Prudente^{1,4}, E. Flex^{1,2}, R. Menghini⁵, M. Federici⁵, V. Trischitta^{1,3,4}, B. Dallapiccola^{1,2}.* 1) IRCCS-CSS Mendel, Rome, Italy; 2) Dept Exp and Pathol La Sapienza Univ Rome Italy; 3) Dept Clinic Sciences La Sapienza Univ Rome Italy; 4) Unit Endocrinology IRCCS-CSS Institute S.G. Rotondo Italy; 5) Dept Internal Med Tor Vergata Univ Rome Italy; 6) Dept Biomed Sciences Univ Chieti Italy.

The genetic background of Type 2 diabetes (T2D) is mostly unknown. Insulin resistance (IR) plays a major role in the pathogenesis of T2D. DIO2 converts thyroid pro-hormone T4 to the active T3 hormone and may play a role in modulating IR. A missense SNP (T92A) of DIO2 has been associated with IR in Caucasians. We genotyped the T92A SNP in 495 T2D and 598 Caucasian controls from Italy. The X92A genotype (i.e. T92A and A92A) was less frequent ($p=0.02$) among T2D (53.1%) than controls (63.7%): OR=0.6 (95% CI=0.4-0.9). The modulation of T2D risk is believed to be polygenic due to multiple gene-gene interactions. Several reports indicate that the P12A missense SNP in the PPAR2 transcription factor plays a role in T2D, with X12A genotype carriers (i.e. P12A and A12A) being at reduced risk. The risk of T2D of DIO2 X92A carriers was significantly reduced in the PPAR2 X12A (OR=0.25 95% CI=0.07-0.8, $p=0.025$) but not in the PPAR2 P12P (OR=0.7 95% CI=0.5-1.2, $p=0.2$) (p for interaction=0.05) group. To investigate the biological significance of this statistical interaction, we performed an EMSA using a radiolabeled oligonucleotide containing a putative binding site for PPAR2 identified in the DIO2 promoter region and in vitro translated PPAR2 with its RXR<ag> partner which is essential for transcriptional activity. A clear shift in the oligonucleotide mobility was observed, thus indicating that the PPAR2/RXR<ag> heterodimer specifically binds to the DIO2 promoter. These data demonstrate the association of the X92A genotype with a reduced risk of T2D in an Italian population. This effect is mediated by interaction with the PPAR2 gene which may be a consequence of specific binding of the PPAR2/RXR<ag> heterodimer to a putative site identified in the DIO2 promoter region.

Association analysis of the functional 1858CT polymorphism within the *PTPN22* gene in several autoimmune diseases. *M.K. Viken¹, S.A. Amundsen¹, A. Smerdel¹, K.M. Boberg², I.M. Gilboe³, V. Lilleby³, T. Kvien³, O. Forre³, L.M. Sollid¹, E. Thorsby¹, B.A. Lie¹.* 1) Institute of immunology, Rikshospitalet University Hospital, Oslo, Norway; 2) Medical Department, Rikshospitalet University Hospital, Oslo, Norway; 3) Department of Rheumatology, Rikshospitalet University Hospital, Oslo, Norway.

The gene *PTPN22* encodes a lymphoid Protein Tyrosine Phosphatase, which plays a role in suppression of T-cell activation. *PTPN22* is localized on chromosome 1p13, a region that shows linkage to rheumatoid arthritis (RA) and weakly to systemic lupus erythematosus (SLE). Recent studies have shown that a single nucleotide polymorphism (SNP) in *PTPN22* is associated with both RA and type 1 diabetes (T1D). The SNP (rs2476601, 1858CT) changes an amino acid and thereby disrupts the binding site for the negative regulatory kinase Csk. Due to the observed association with the two autoimmune diseases T1D and RA displaying different phenotypes, we wanted to investigate if this 1858CT SNP could be associated with autoimmune diseases in general. To test this hypothesis, we genotyped (by TaqMan assay, Applied Biosystems) several autoimmune diseases using case-control materials from the Norwegian population. The data sets consist of patients with primary sclerosing cholangitis (PSC; n=238), juvenile rheumatoid arthritis (JRA; n=319), SLE (n=162), celiac disease (CD; n=313) and controls (n=555). Given the minor allele frequency of 0.116 and the odds ratio of 1.79 previously reported in T1D, our study has the following power: 0.85 for PSC, 0.9 for JRA, 0.75 for SLE and 0.9 for CD. In SLE and PSC we did not observe any signs of association, while we found an association for carriers of the risk allele versus non-carriers in both JRA (OR=1.35; p=0.04) and CD (OR=1.34; p=0.04). We are currently testing the SNP in a Norwegian RA material (n=~600) in an effort to confirm the association already found in RA, thus placing our results in a broader perspective. In conclusion, the *PTPN22* 1858CT SNP may be a risk factor for several autoimmune diseases.

Significant association between PTPN22 gene polymorphism and type 1 diabetes in Newfoundland. *K.S. Wang¹, M. Liu¹, B. Bharaj¹, M. Lu¹, H.T. Chen¹, J.A. Curtis³, L.A. Newhook³, A.D. Paterson^{1,2}.* 1) Program in Genetics and Genomic Biology, The Hospital for Sick Children, Toronto; 2) Dept. of Public Health Sciences and Psychiatry, Univ. of Toronto; 3) Pediatrics, Memorial Univ. of Newfoundland, Canada.

PTPN22 encodes the lymphoid protein tyrosine phosphatase (LYP), a suppressor of T-cell activation that maps to 1p13. A SNP (rs2476601) in the PTPN22 gene has been reported to be associated with type 1 diabetes (T1D) using case-control studies in both North American and Italian samples (Bottini et al. 2004). We used the family based association test (FBAT) for an association study between the same SNP and T1D using DNA from 625 persons with T1D and 1532 relatives without diabetes from 834 nuclear families recruited from Newfoundland (NF) where there are documented founder effects and a high incidence of T1D. The TDTPHASE program was used to compare maternal and paternal transmissions as well as gender specific transmissions in probands because the risk of T1D may differ between males and females and some T1D susceptibility loci may be subject to genomic imprinting (i.e. the parental origin of a susceptibility allele affects penetrance). The minor allele frequency in probands was 0.17 and the locus was in Hardy-Weinberg equilibrium. FBAT showed that the minor allele was transmitted more often than expected to affected offspring ($Z=3.003$, $P=0.0027$). TDTPHASE indicated that the minor allele revealed significant transmission disequilibrium from parents to affected offspring ($P=0.00093$) but not to unaffected offspring ($P=0.43$). Interestingly, there was a significant difference between transmission of the minor allele to male and female affected offspring ($P=0.023$). In addition, the paternal allele showed significant transmission to affected male offspring ($P=0.00098$) compared to affected female offspring ($P=0.90$), while the same maternal allele was not associated in families with affected male or female offspring. In summary, our results confirm the previous report of a significant association between PTPN22 gene polymorphism and T1D, especially we find evidence for gender differences and parental original effects.

Tyrosine phosphatase polymorphism C1858T associates with type 1 diabetes but not with diabetic nephropathy.

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Type 1 diabetes (T1D) is a significant medical problem worldwide with the highest incidence rate of 45/100,000 per year in Finland. T1D arises from an autoimmune destruction of the pancreatic β -cells leading to a complete dependence on exogenous insulin. About 30% of T1D individuals develop diabetic nephropathy, a condition characterized by increase in the arterial blood pressure, continuous albuminuria, and relentless decline in renal function. The functional SNP C1858T of the protein tyrosine phosphatase, nonreceptor-type 22 gene (*PTPN22*) encoding the lymphoid-specific intracellular phosphatase (LYP) was recently associated with T1D in North American and Sardinian populations. LYP is among the most powerful inhibitors of T-cell activation and thus a candidate for autoimmune diseases. Our aims were to study the role of this SNP in T1D and diabetic nephropathy in clinically well-defined Finnish T1D patients (n=1277). The T-allele was more frequent in T1D patients than in healthy subjects (n=184; p=0.000014). However, no association with diabetic nephropathy was detected. The allele frequencies in various sub-populations were almost identical, as well as the odds ratios for the T-allele carriers. A weak association observed between the T-allele and thyroxin treatment, an indicator for hypothyreosis, might imply the role of the C1858T variant in other autoimmune diseases. In conclusion, our results support the original finding showing that the functional variant C1858T resulting in an Arg620Trp substitution is associated with T1D. This variant might have a real impact on the pathogenesis of T1D since the association has now been detected in three different populations.

Screening of SNPs previously reported to be associated with type 2 diabetes in a Finnish population. *C.J. Willer¹, L.L. Bonnycastle², E. Smith², A.U. Jackson¹, A.D. Skol¹, K.L. Mohlke^{2,3}, L.J. Scott¹, N. Narisu², P.S. Chines², M.R. Erdos², R.N. Bergman⁴, J. Tuomilehto⁵, F.S. Collins², M. Boehnke¹.* 1) Department of Biostatistics, University of Michigan, Ann Arbor, MI; 2) Genome Technology Branch, National Human Genome Research Institute, Bethesda, MD; 3) Department of Genetics, University of North Carolina, Chapel Hill, NC; 4) Department of Physiology and Biophysics, Keck School of Medicine, University of Southern California, Los Angeles, CA; 5) Diabetes and Genetic Epidemiology Unit, Department of Epidemiology and Health Promotion, and Department of Biochemistry, National Public Health Institute, Helsinki, Finland and Department of Public Health, University of Helsinki, Helsinki, Finland.

The Finland-United States Investigation of NIDDM Genetics (FUSION) study aims to identify genetic variants that influence susceptibility to type 2 diabetes and heritable variability in diabetes-related quantitative traits. We are undertaking an association study of a large number of candidate genes to complement other gene localization strategies we have employed in regions of linkage. The latter was successful in the identification of an association with the HNF4-alpha P2 promoter region ($p = .01$, OR = 1.34, CI = 1.07 - 1.66), which we have previously published. We performed a comprehensive literature search to survey previously reported associations between type 2 diabetes status and single nucleotide polymorphisms (SNPs). We found reports of significant associations with 106 SNP markers in 55 unique genes. Of the 106 SNPs identified, we have to date analyzed 19 SNPs for association in 795 cases and 426 controls. We identified one positive association with PPAR-gamma ($p = .001$, OR = 0.65, CI = 0.52 - 0.82) which we have reported previously. Four of the remaining SNPs were excluded because of difficulty mapping their position. Currently we are genotyping the remaining 83 SNPs in 790 cases and 650 controls using high-throughput genotyping by bead array (Illumina) or by MALDI-TOF (Sequenom). We will present analyses of association in 102 SNPs to assess replication of previously published associations with type 2 diabetes mellitus.

SHH, SIX3, and TGIF in holoprosencephaly- mutations, phenotype and image. How different they are? L.

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Holoprosencephaly (HPE) is the most common developmental field defect resulting from a failure of midline division of the forebrain, it is etiologically heterogeneous, pathogenetically complex, and phenotypically variable. Abnormal forebrain cleavage results in three particular types of HPE: lobar, semilobar, and alobar, however, ethmocephaly, cebocephaly, premaxillary agenesis, absence of olfactory bulbs and tracts, structural anomalies of the corpus callosum, bilateral cleft lip/palate, single central incisor and hypotelorism compose a wide mosaic with each one of these findings nesting per se a holoprosencephalic phenotype. Different genes are implicated in the pathogenesis of HPE; these include SHH, ZIC2, SIX3, and TGIF, most of the cases are isolated and phenotypical manifestations are variable in each one. A distinctive phenotype was reported in patients with GLI2 mutations whose primary features include defective anterior pituitary formation and pan-hypopituitarism, with or without overt forebrain cleavage abnormalities, midfacial hypoplasia, isolated hypotelorism, solitary median maxillary central incisor, cleft lip/palate, and mid-face hypoplasia, leading to the so-called HPE-like phenotype, however, up to now there is no standard definition of this phenotype. We have ascertained a sample of 50 isolated HPE patients, 27 with typical HPE phenotype and 23 with the HPE-like phenotype. Mutational analysis and neuroimage evaluation was performed in all patients. SHH mutations were detected in 4 patients with HPE-like with normal MRI and in 1 patient with semilobar HPE. From the patients with SIX3 mutations 5 presented alobar HPE and 1 semilobar. The patient with TGIF mutation presented HPE-like phenotype, structural anomalies of the corpus callosum and increased size of IIIrd and IVth ventricles. Our data suggest that mutations involving the SIX3 gene are more deleterious in relation to the developing central nervous system than the genes involving the SHH signaling cascade since it is essential for the development of the anterior neural plate and eye in humans.

Mutations in steroid 5-alpha reductase type 2 and the severity of hypospadias. *J. Manson, M. Carr.* Dept Human Genetics and Pediatric Urology, Children's Hosp Philadelphia, Philadelphia, PA.

The purpose of this project is to evaluate gene-environment interactions in the risk for hypospadias. Our hypothesis is that allelic variants in genes controlling androgen action and metabolism (steroid 5-alpha reductase type 2 [SRD5A2] and the androgen receptor [AR]) will be highly associated with the risk for and severity of hypospadias. Parental exposure to environmental agents during pregnancy may further increase the risk for hypospadias in fetuses with a susceptible genotype, resulting in a gene-environment interaction. This is the first study in a large, outbred population to investigate gene-environment interactions on the risk of this birth defect. Families of case infants less than one year of age presenting for diagnosis/surgical repair of hypospadias in a Pediatric Urology clinic are recruited into the study. Families of affected control infants with renal anomalies are recruited from the same population. Parents are administered questionnaires to obtain information on reproductive and obstetrical history and exposures to drugs and environmental agents. Buccal swabs are collected from the mother, father and infant and DNA extracted for evaluation of candidate genes. Results to date on approximately 350 families indicate that there are no differences between groups for maternal or paternal occupational exposures, while case mothers reported significantly more exposures to paints and stains and case fathers to pesticides at home than their respective controls. The most common genotype finding in the study population was a V89L missense mutation in exon 1 of the SRD5A2 gene. There was a highly significant association between severity of hypospadias and the frequency of this mutation. There were no differences between case and control groups for CAG and GGC repeat lengths on the androgen receptor. The V89L mutation in the SRD5A2 gene is known to decrease activity of the enzyme by approximately 30%. To date, there is no significant gene-environment interaction between this mutation and environmental exposures.

MAOA gene polymorphisms in obsessive-compulsive disorder: Analysis of gender differences. *J.M. Geronimo, P.D. Arnold, M. Lanktree, M.A. Richter, J.L. Kennedy.* Neurogenetics, Centre for Addiction and Mental Health, Toronto, Ontario, Canada.

Obsessive-Compulsive Disorder, OCD, is a common and severe psychiatric illness that affects 1-3% of the population; it is characterized by recurrent, unwanted thoughts and/or repetitive behaviors. Some studies have reported that onset of the disorder is earlier for males (6-15 years versus 20-29 for females), and that OCD males and females are characterized by different symptoms. Thus, the clinical and biological heterogeneity of OCD suggest that the etiology of OCD may have a sex-linked component. One candidate gene on the X chromosome that is of particular interest for OCD is the gene for monoamine oxidase A (MAOA), since this MAO isozyme plays an important role in the degradation of the neurotransmitters norepinephrine, epinephrine, dopamine, and serotonin. In the current study, the MAO-A polymorphisms investigated were a 23-base-pair variable number of tandem repeats (VNTR) and a restriction fragment length polymorphism (RFLP) resulting from a single-base-pair substitution at MAO-A Exon8 (G297T).

These polymorphisms were analyzed in 68 OCD families including trios and families with at least one sib pair, of which 32 probands were male and 36 were female. Analysis using the Family Base Association Test (FBAT) showed preferential transmission of the four repeat VNTR allele in males ($z=2.14$, $p=0.03$) and no statistically significant association for females. However, in our total sample no statistically significant genotypic or allelic associations were detected for the VNTR (allele 4: $z=1.24$, $p=0.22$ and allele 3: $z=-1.01$, $p=0.31$) and Exon8 polymorphisms ($z=0.35$, $p=0.737$). Results were not corrected for multiple comparisons. These preliminary results suggest a sex-specific association of the MAOA VNTR, consistent with earlier studies of MAOA variants in OCD. Further investigation in larger samples is warranted.

Analysis of maternal smoking and sixteen detoxification genes in the in the etiology of orofacial clefts. *M. Shi*¹, *C. Christensen*², *C. Weinberg*³, *A. Lozada*⁴, *P. Romitti*⁵, *J.C. Murray*^{1,4,5}. 1) Dept Biological Sci, Univ Iowa, Iowa City, IA; 2) Center for the Prevention of Congenital Malformations, Institute of Public Health, Univ Southern Denmark, 5000 Odense C, Denmark; 3) Biostatistical Branch, National Institute of Environmental Health Sciences; 4) Dept Pediatrics, Univ Iowa, Iowa City, IA; 5) Dept Epidemiology, Univ Iowa, Iowa City, IA.

We extended our previous work on the investigation of potential interaction effects between maternal cigarette smoking and variants in smoking detoxification genes and risk of orofacial clefts. DNA samples from families with cleft cases as well as unaffected control families came from two population-based studies, one in Denmark and one in Iowa (US). A total of 3008 Danish and 2494 Iowa DNA samples were used in the study, out of which 1257 were case samples (including 823 complete case/parent triad sets). Genotyping was carried out on 25 single nucleotide polymorphisms in 16 genes involved in phase I and phase II detoxification pathways. Effects of maternal and fetal genotypes as well as the interaction between these genotypes and maternal smoking were tested. Statistical tests were also carried out to examine the interaction between the genes involved in the detoxification pathway as well as genes important for palatal embryonic development, such as *MSX1*, *TGFA* and *IRF6*. Logistic regression and log-linear modeling were applied in the analysis. Preliminary analysis showed statistically significant interaction effects between maternal smoking and the *EPHX1* T113Y polymorphism in Iowa samples and was suggestive of possible interaction effects between smoking and two of the *GST* genes, *GSTT1* and *GSTP1*. The analysis is now being extended to a recently acquired collection of 285 pentads consisting of the case, both parents and the maternal grandparents allowing for more specific characterization of maternal effects. This study will help us in understanding the possible roles played by gene-environment interaction as well as gene-gene interaction in the etiology of orofacial clefts.

Mutant ataxin-7 induced neurodegeneration in SCA7 transgenic mice. *L.A. Duvick, M.D. Kaytor, R.G. Ehlenfeldt, M.S. Berlinger, H.B. Clark, H.T. Orr.* Lab Medicine and Pathology, Institute of Human Genetics, Univ of Minnesota, Minneapolis, MN.

Spinocerebellar ataxia type 7 (SCA7) is an autosomal dominant neurodegenerative disorder caused by an expansion of a polyglutamine repeat within the *SCA7* encoded protein, ataxin-7. To gain insight into the molecular basis of Purkinje cell degeneration observed in this disease, the Purkinje cell specific promoter (*Pcp-2*) was used to over-express *SCA7* with 110 CAG repeats. Previous models of *Pcp-2 SCA7* transgenic mice failed to exhibit a phenotype and pathology until late in age. This was most likely due to low levels of ataxin-7 expression due to position integration effects of the transgene. To circumvent this, the transgene was inserted between insulator sequences from the chicken *-globin* gene. Insulators are regulatory elements that have been shown to protect gene expression from both positive and negative effects of chromatin at the insertion of a transgene. Four founder animals were identified that gave rise to lines that expressed ataxin-7 protein uniformly in the nucleus of Purkinje cells in the cerebellum. Staining of ataxin-7 in the cerebellum of transgenic mice showed regional differences in protein accumulation and pathology. At early time points (6 wks), ataxin-7 was diffusely nuclear but gradually accumulated into single large nuclear inclusions (NIs) in an anterior to posterior manner. Purkinje cell neurons that form large NIs seemed to be spared while cells that retained a diffuse staining pattern showed signs of neurodegeneration. This was quantified by measurement of the molecular layer, and number of cytoplasmic vacuoles in Purkinje cells in different regions of the cerebellum. Moreover these animals had a behavioral deficit by rotorod assessment at 12 weeks of age. This animal model of SCA7 further supports the idea that the formation of NIs is neuroprotective in polyglutamine disease.

Modulation of ataxin-1 phosphorylation. *M.D. Kaytor*¹, *C.E. Byam*¹, *S.D. Stevens*¹, *H.Y. Zoghbi*², *H.T. Orr*¹. 1) Dept Lab Medicine and Pathology, Institute of Human Genetics, Univ of Minnesota, Minneapolis, MN; 2) Howard Hughes Medical Institute, Dept Molecular and Human Genetics, Baylor College of Medicine, Houston TX.

Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant neurodegenerative disorder caused by the expansion of a glutamine repeat within the *SCA1*-encoded protein ataxin-1. We have previously shown that serine 776 (S776) of both wild type and mutant ataxin-1 is phosphorylated *in vivo* and *in vitro*. Moreover, S776 of ataxin-1 was shown to play a critical role in SCA1 pathogenesis. Preventing phosphorylation of this residue by replacing it with alanine resulted in a mutant protein found in the nucleus that was not pathogenic. To further investigate the biological function of phospho-S776 ataxin-1 we have developed a cell-culture based assay to screen for modulators of S776 phosphorylation. In this assay, ataxin-1 expression is monitored by EGFP fluorescence in cell lines stably expressing an EGFP-ataxin-1 fusion protein. The previously developed and characterized phospho-S776 ataxin-1 specific antibody (PN1168) is then used to assess ataxin-1 S776 phosphorylation. We are currently screening a library of 84 known kinase and phosphatase inhibitors. An initial screen of this library at a concentration of 25M identified 17 compounds capable of either increasing or decreasing the relative level of phospho-S776 ataxin-1 by at least 35%. Preliminary analysis of these hits places many of the inhibited kinases in a common pathway leading to ataxin-1 S776 phosphorylation. We are currently verifying the specificity and efficacy of these results by re-screening the library at a lower concentration of inhibitor. We have also begun to screen a library of 2000 drugs and natural products to identify additional modulators of ataxin-1 S776 phosphorylation. Screening for compounds that alter the phosphorylation of ataxin-1 will identify new molecular tools to aid in elucidating the mechanism of SCA1 pathogenesis. Moreover, identified compounds will provide potential leads toward the development of a therapeutic treatment for SCA1.

Genetic background modifies early disease events in the striatum of Huntingtons disease knock-in mice. A. Lloret, E. Janice, A. Teed, M. Hakky, M.E. MacDonald, V.C. Wheeler. Molecular Neurogenetics, Mass. General Hospital, Charlestown, MA. .

Huntingtons disease (HD) is a dominant neurodegenerative disorder caused by the expansion of a polymorphic CAG repeat that elongates a glutamine tract in huntingtin (Htt). Mutant Htt triggers the demise of vulnerable medium spiny neurons in the striatum and the onset of motor symptoms. Although the underlying process that determines disease onset is strongly CAG length-dependent, modifying factors have been implicated by variability in onset age not accounted for by CAG size. Studies of the *HD* mutation in *Hdh* knock-in mice have shown that long CAG repeats that lengthen the glutamine tract in murine huntingtin initiate a striatal-specific disease process. We have characterized a number of early striatal phenotypes in *Hdh*^{Q111} knock-in mice on an outbred CD1 background, as well as the striatal-specific time-dependent somatic expansion of the *Hdh*^{Q111} CAG repeat. To identify genetic modifiers of early disease phenotypes in *Hdh*^{Q111} knock-in mice we have generated congenic lines by repeated backcrossing of the *Hdh*^{Q111} allele onto three inbred genetic backgrounds, C57BL/6, 129Sv and FVB. We have investigated an early phenotype, the nuclear accumulation of mutant huntingtin, as well as the somatic instability of the CAG repeat in the striatum of these three lines. Quantitation of nuclear mutant huntingtin accumulation at 6 months of age revealed statistically significant differences between all three lines; C57BL/6 mice had the highest, 129Sv the lowest, and FVB an intermediate level. Nuclear mutant huntingtin levels on the C57BL/6 and 129Sv backgrounds were non-overlapping. Analyses of somatic instability in the three congenic lines indicate reduced levels of somatic expansion in 129Sv mice compared to the C57BL/6 and FVB strains. These data imply the presence of gene(s) that modify an early striatal phenotype and somatic expansion of the *HD* CAG repeat in *Hdh*^{Q111} knock-in mice. We are currently carrying out genetic crosses between the strains to determine the heritability of the genetic modifier(s).

Investigating a possible relation between candidate genes involving retinoid function and psychosis. *N.A. Akarsu¹, B. Akin¹, H. Okur¹, S. Ozer², T. Tuncali³, A. Gogus², A.B. Goodman⁴, A. Ulusahin².* 1) Gene Mapping Lab, Dept Pediatrics and; 2) Dept Psychiatry, Hacettepe Univ, Ankara, Turkey; 3) Dept Med Genet, Ankara Univ, Ankara, Turkey; 4) Massachussets Mental Health Center, Harvard Medical School, Boston MA.

Recent studies supported the continuum model of psychosis suggesting that transmission of psychosis is not specific to bipolar disorder or schizophrenia (SCH). We previously presented an inbred Turkish family with the continuum model (*Prog. Neuro-Psychopharmacology and Biol. Psychiatry* 2004;28: 255). Of 40 members examined, 18 had psychosis. The coexistence of rickets-alopecia syndrome (OMIM 277440) and psychosis in the same pedigree led us to investigate presumed relationship between vitamin D deficiency and psychosis. Linkage analysis excluded VDR locus on chromosome 12q13. We subsequently tested the additional hypothesis that retinoid dysregulation may be important in the etiology of SCH (*PNAS*; 1998;95;7240). A total of 57 DNA markers flanking or within the following loci were studied: RARA at 17q; RARB and PPARG at 3p; RARG at 12q; RXRA at 9q; DTNBP1, RXRB and PPARD at 6p; CRABP2 and RXRG at 1q22; DISC1 at 1q42; NURR1 at 2q; entire chromosome 22 containing PRODH, COMT, GRK3, APOL (4,2,1), and PPARA genes; LPL, NRG1, PPP3CC at 8p; DRD3, RBP1/RBP2 at 3q and DRD2, 5HT3 at 11q. Results from initial screening panel with a total of 12 affected cases were included in this study. A model-based approach was selected to perform linkage analysis. The individuals with a psychosis phenotype (SCH, schizoaffective disorder, bipolar and unipolar affective disorder with psychotic features) were regarded as affected. The parameters used in both simulation (SLINK) and linkage analysis were: The penetrance for gene carriers: 0.40 and for nongene carriers: 0.003, mutant allele frequency of 0.01 which reflects population prevalence 0.02 with 0.27 phenocopy rate. Positive lod scores were only obtained in RXRA, DTNBP1 and RXRG regions assuming autosomal dominant inheritance. Allele loss was observed in some family members in the DNA markers D6S1959 and D1S1679 (from DTNBP1 and RXRG locus respectively). Supported by NARSAD; Akarsu-2003II.

Analysis of the 9p22.3-22.1 region in Italian families with allergic asthma. *A. Beghini, G. Malerba, C. Bombieri, P.F. Pignatti.* Department of Mother and Child, Biology and Genetics, University of Verona, Verona, Italy.

We previously reported a genome scan for asthma in 123 Italian families. The following 4 phenotypes were studied: clinical asthma, total serum elevated IgE (IgE), skin prick test positivity to common aeroallergens (SPT) and bronchial hyperresponsiveness to methacholine (BHR). Markers on chromosome 9p22.3-22.1 (D9S285 and D9S157) showed suggestive linkage for IgE and SPT. The 19 families that presented null or positive linkage for SPT or IgE were selected for an association study by the transmission disequilibrium test (TDT). Five SNPs (single nucleotide polymorphisms) flanking the D9S285 and D9S157 markers were chosen. The SNPs have been selected from data base dbSNP (<http://www.ncbi.nlm.nih.gov>), Ensembl (<http://www.ensembl.org>) and SNPper (<http://snpper.chip.org>). Two SNPs, 751804 T/C and 2275256 G/A, were polymorphic in 20 unrelated subjects. The genetic analysis technique used was ddNTP Primer Extension or Minisequencing. The individuals of the 19 families were genotyped for the 2 SNPs with the SNaPshot Multiplex Kit (Applied Biosystems). The reaction products are analyzed on ABI PRISM 310 DNA sequencer (Applied Biosystems) and with the Genescan software. The observed frequency of the minor allele was 14% and 35% for SNP 751804 and SNP 2275256, respectively. TDT did not show any significant association for either SNP. Multilocus analysis showed a preferential transmission of haplotype TA for asthma ($p = 0.007$), IgE ($p = 0.0036$), SPT ($p = 0.0046$) and BHR ($p = 0.03$). These data suggest that a still unknown genetic factor might be located in the region. Other SNPs in the region have been selected for a finer localisation.

A comprehensive linkage analysis of putative bipolar affective disorder loci. *H. Xu*^{1,2}, *R. Cheng*², *J. Liu*², *J. Endicott*³, *J.E. Loth*³, *T.C. Gilliam*^{1,2}, *M. Baron*³. 1) Department of Genetics and Development, Columbia Univ, New York, NY; 2) Columbia Genome Ctr, Columbia Univ, New York, NY; 3) Department of Psychiatry, Columbia University, New York, NY.

Bipolar affective disorder (BP), also known as manic-depression, is a severe and common psychiatric disorder with complex etiology. Family, twin and adoption studies have implied a significant genetic component underlying the disease. However, the susceptibility genetic factors remain elusive. In a previous genome-wide scan of 343 microsatellite markers (average distance ~10 cM) in 40 multiplex pedigrees (373 genotyped individuals), we reported several potential loci for BP (J. Liu et al., 2003). In the present follow-up study, we have sought to narrow down the putative BP-linked regions for susceptibility gene identification. To this end, we saturated the 11 regions implicated in the genome-wide scan with 87 densely mapped microsatellite markers (average distance ≤ 2 cM) flanking the original linkage peaks. The sample genotyped included the original, extended pedigrees and an additional 16 multiplex families (a total of 862 individuals in 56 pedigrees). This is one of the largest and well-characterized samples reported to date. The 11 regions included in this study are 2p13-16, 3q27-28, 4q31, 8q13, 9q31, 10q21, 10q24, 17q11-12 (two-point parametric LOD scores ≥ 1.9), and 7q34, 13q32 and 14q21 (multipoint ASP LOD scores > 2). Linkage findings in some of these regions were also suggested by independent studies from other groups. Linkage analysis of the densely mapped genomic regions is underway, and the findings will be reported at the time of the presentation. For the genomic regions with verified linkage to BP in this comprehensive mapping, high-density SNPs across the linked regions will be genotyped for further elucidation of susceptibility genetic factors.

Chromosome 5q candidate genes in celiac disease: no evidence for the involvement of IL4, IL5, IL9, IL13, IL17B and NR3C1. *A.W. Ryan^{1,3}, J.M. Thornton^{1,3}, K. Brophy^{1,3}, J.S. Daly^{1,3}, C. O Morain¹, R.M. McLoughlin¹, N.P. Kennedy¹, M. Abuzakouk², F.M. Stevens⁴, C. Feighery², D. Kelleher^{1,3}, R. McManus^{1,3}.* 1) Clinical Medicine, Trinity College, Dublin, Ireland; 2) Dept. of Immunology, Trinity College, Dublin, Ireland; 3) Dublin Molecular Medicine Centre, Dublin, Ireland; 4) Department of Medicine, National University of Ireland, Galway.

Celiac disease (CD) is a complex autoimmune disorder of the small intestine, brought about by the ingestion of dietary gluten and related proteins in susceptible individuals. Genetic predisposition to CD is determined primarily by alleles at the HLA-DQB locus, while other MHC linked genes (6p21) and CTLA4 (2q33) have also been implicated. In addition, extensive family studies have provided strong evidence for a susceptibility locus on chromosome 5q. However, the position of maximum marker association to CD on 5q has varied considerably among family studies, and the causative gene has not yet been identified. We have assayed genetic variation at 13 single nucleotide polymorphism (SNP) loci from IL4, IL5, IL9, IL13, IL17B and NR3C1, all of which are present on chromosome 5q and have potential or demonstrated involvement in autoimmune and/or inflammatory disease, in a sample of 409 CD cases and 355 controls. SNPs were chosen on the basis of functional relevance, prior disease association and, where possible, prior knowledge of haplotype variation in European populations (haplotype tagging). The frequencies of the most common haplotypes in cases and controls respectively were: IL4/IL13, 0.665 vs. 0.691; IL5, 0.660 vs. 0.668; IL9, 0.406 vs. 0.437; IL17B, 0.492 vs. 0.483; NR3C1, 0.639 vs. 0.624. The frequencies of these (and the rarer) haplotypes were, therefore, similar in cases and controls. These results, which incorporate almost ten thousand genotypes from ethnically uniform population samples, provide good evidence that these loci are not associated with CD in this sample population. In addition, no evidence for the involvement of candidate genes IL12B and IBD5 has been provided by earlier studies. Further candidate gene regions for the elusive chromosome 5q CD susceptibility locus are currently under investigation.

Molecular genetic study of Apolipoprotein L gene family at 22q11-13 as candidates for schizophrenia. M.-C.

*Cheng*¹, *W.-H. Chien*², *H.-M. Liao*¹, *C.-H. Chen*^{1,3}. 1) Department of Psychiatry, Tzu-Chi Univ, Hualien, 970 Taiwan; 2) Institute of Aboriginal Health, Tzu-Chi Univ, Hualien, 970 Taiwan; 3) Institute of Human Genetics, Tzu-Chi Univ, Hualien, 970 Taiwan.

Background: Several genome-wide linkage and molecular genetic studies suggested that chromosome 22q11-13 is one of the susceptible loci for schizophrenia. Microarray analysis of postmortem brain of patients with schizophrenia revealed up-regulation of Apolipoprotein L (APOL) family gene expressions in the prefrontal cortex of schizophrenia. Since the APOL family genes are located at chromosome 22q11-13, we proposed that APOL gene family might be candidate genes for schizophrenia. Methods: To test this hypothesis, we systematically searched the APOL family genes for mutations at protein-coding sequences and promoter region using Denaturing High Performance Liquid Chromatography (DHPLC) and autosequencing. Results: We identified four, two, eight, twenty one, and four mutations in APOL1, 2, 3, 4, 5 genes, respectively. Several of these mutations result in missense mutations including E150K at exon 7 of APOL1; I245V at exon 6 of APOL2; S39R and V232M at exon 3 and exon 7 of APOL3, respectively; M156V, R220H, A316Q, and S323L at exon 8 of APOL4; M272T and M323T at exon 3 of APOL5. In addition, a GT to GG substitution was identified at conserved donor splicing site of intron 3 of the APOL4. Discussion: These mutations might affect the function of APOL family genes and cause pathology of schizophrenia. Currently, we are conducting a case-control study to test the association of these mutations with schizophrenia.

Localization of an Alcohol Dependence gene to the 5q GABAA cluster. *M. Radel¹, R. Vallejo¹, N. Iwata², R. Aragon¹, J.C. Long¹, M. Virkkunen³, D. Goldman¹.* 1) Laboratory of Neurogenetics, NIAAA, NIH, Rockville, MD; 2) Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Japan; 3) Department of Psychiatry, University of Helsinki, Finland.

Background: Behavioral and pharmacogenetic evidence links gamma-amino butyric acid type A (GABAA) receptors and chromosomal regions containing GABAA receptor genes to ethanol-related responses. The GABAA cluster on chromosome 5q is of particular interest in the genetics of Alcohol Dependence (AD) because of the gamma-2 subunit requirement for ethanol modulatory action on GABAA receptors, and due to previous linkage findings in mice and humans implicating both GABRA6 and GABRG2, and reported associations of GABRA6, GABRB2 and GABRG2 alleles with AD. Methods: Two large psychiatrically interviewed samples, a Southwestern Native-American (SWNA) sample (total n = 433) and a Finnish sample (total n = 511) with alcohol-dependent subjects and unaffected individuals were genotyped blind to diagnosis for six single nucleotide polymorphisms at the 5q GABAA gene cluster. Sib-pair linkage and case-control association analyses were performed in addition to linkage disequilibrium mapping to investigate the role of these genes in susceptibility to AD. Results and conclusions: Sib-pair linkage of GABRG2 to AD was observed in Finns ($P = 0.008$). Association of the GABRB2 1412T allele with AD was detected in both Finns ($P = 0.01$), and in the SWNA tribe ($P = 0.008$), and the GABRA6 1519T allele was associated with AD in both Finns ($P = 0.01$) and the SWNA tribe ($P = 0.03$). Linkage disequilibrium mapping with haplotypes yielded evidence for an AD predisposition locus in the region of the GABAA 5q gene cluster in both populations. For Finns the most highly significant findings were at GABRA6 (empirical $P = 0.007$) and the region spanning from the GABRA6 1519T>C polymorphism to the GABRG2 IVS8 +99C>A polymorphism (empirical $P = 0.05$). For the SWNA tribe the most highly significant signals were also at GABRA6 (empirical $P = 0.04$) and the region spanning from the GABRA6 1519T>C polymorphism to the GABRG2 IVS8 +99C>A polymorphism (empirical $P = 0.05$). In both populations, haplotype localization implicates GABRA6 and GABRG2.

Effects of gene interactions on male fertility. *W. Engel¹, B. Drabent², A. Meinhardt³, I. Adham¹, I. Schwandt¹, S. Wolf¹, U. Sancken¹, K. Kleene⁴, K.C. Nayernia¹.* 1) Inst Humangenetik, Univ Gottingen, Gottingen, Germany; 2) Department of Biochemistry, University of Gttingen, 37073 Gttingen, Germany; 3) Department of Anatomy and Cell Biology, University of Giessen, 35378 Giessen, Germany; 4) Department of Biology, University of Massachusetts, Boston, Massachusetts.

About 15% of couples have reduced fertility and in approximately one-half of all cases the reason is male infertility, usually of genetic origin. It is obvious that the molecular basis of male (in)fertility is not a linear order of genetic events but the interaction of complex genetic networks. Triple knockout mice were used to investigate the interactions of five genes that are expressed in meiotic and haploid spermatogenic cells in mice, transition protein 2 (Tnp2), proacrosin (Acr), histone H1.1 (H1.1), histone H1t (H1t) and sperm mitochondria-associated cysteine-rich protein (Smcp). TNP2 functions in the replacement of histones and the initial condensation of the spermatid nucleus. The linker histone subtypes H1.1 and H1t are expressed at high levels in meiotic and early haploid cells. ACR, a protease that is stored as a proenzyme in the acrosome, is activated during the acrosome reaction and functions in binding of sperm to the zona pellucida. SMCP is a structural protein in the outer membranes of sperm mitochondria that functions in motility. Previous work demonstrates that homozygous knockout mice lacking each of these proteins individually exhibit no defect in fertility on mixed genetic backgrounds. In contrast, the present study demonstrates that five triple knockout lines, *Acr/H1.1/Smcp*, *Acr/Tnp2/Smcp*, *Tnp2/H1.1/Smcp*, *Acr/H1t/Smcp*, *Tnp2/H1t/Smcp* exhibit drastic reductions in fertility on mixed genetic backgrounds. Analysis of fertility parameters reveal that the decreased fertility is due to line-dependent defects in sperm motility in vitro correlated with reduced migration in the female reproductive tract, and decreased fertilization due to defects in adhesion of sperm to the zona pellucida, the membrane surrounding the egg. These findings demonstrate that male fertility involves synergistic interactions of genes that function in sperm motility and sperm-egg adhesion during fertilization.

Childhood-Onset Mood Disorder: Haplotype Analysis of Tryptophan Hydroxylase 1 and 2 Polymorphisms and Suicide Attempts. *J. Strauss*¹, *N. Bulgin*¹, *N. King*¹, *C. George*², *S. Shaikh*¹, *M. Kovacs*², *J.L. Kennedy*¹. 1) Neurogenetics Section, CAMH, Toronto, ON, Canada; 2) Western Psychiatric Institute and Clinic, University of Pittsburgh Medical Center, Pittsburgh, PA.

As part of a study of risk factors for childhood-onset mood disorder (COMD), we examined tryptophan hydroxylase markers for association with suicidal behaviour, a common feature of depressive illness. Observations that tryptophan hydroxylase 2 (TPH2) is expressed in murine brain, while tryptophan hydroxylase 1 (TPH1) is not (Walther et al. *Science* (2003)299:76) have been made. Evidence points to the same differential expression in humans (Zill et al. *Eur Neuropsychopharmacol* (2004)14:284). TPH1 variants have been associated with suicidal behaviour and completed suicide in multiple studies. We investigated the -6526G>A and A218C polymorphisms of TPH1 and the hCV245410, rs1487280 and rs1872824 variants of TPH2 for association with suicide attempts in a sample of 176 subjects with onset of major depressive disorder or dysthymic disorder prior to the age of 14 (97 with attempts, 79 without). Allelic chi-square results indicated no association of the three TPH2 markers in COMD probands with at least one lifetime suicide attempt ($0.70 < p < 0.96$). At the TPH1 locus, the -6526G>A polymorphism was not associated with lifetime history of suicide attempt ($p=0.80$), though the A218C variant exhibited a trend towards association ($\text{chisq}=3.03$, $\text{d.f.}=1$, $p=0.082$). PHASE v2.0 (Stephens et al. 2001) was used to examine haplotypes. Haplotype analysis demonstrated no significant differences between cases and controls for the four TPH1 haplotypes ($p=0.91$) or for the eight TPH2 haplotypes ($p=0.76$). To summarize, we found no association between TPH2 alleles and COMD subjects with suicide attempts. Instead, we remarked a trend for the TPH1 A218C marker to be associated with suicide attempts in this sample. No significant haplotype associations were seen. Our results are part of an emerging understanding of TPH and suicidal behaviour, and are interesting given the TPH isoforms' differing tissue expression.

Analysis of polymorphisms Bsm I, Apa I and Taq I in the Vitamina D Receptor gene (VDR) in patient with Osteoporosis in Venezuela. *L. Borjas-Fajardo, M. Zambrano, L. Pineda, E. Fernandez, P. Montiel, W. Zabala, MG. Portillo, MA. Sanchez, A. Morales, JA. Chacín, W. Delgado.* Unidad de Genetica Medica, Univ del Zulia, Maracaibo, Zulia, Venezuela.

The osteoporosis (OP) is a systemic and heterogeneous skeletal disease characterized by a low density of bony mass. Numerous studies have demonstrated that the genetic factors could represent 80-90% of the variability in the bone mineral density. One of the genes more studied in this field it is the receiving gene of the vitamin D (RVD). In this investigation it was carried out the analysis of the polymorphisms Bsm I, Apa I and Taq I of the gene RVD in a sample of 171 women, 76 normal women (group), 24 with osteopenia and 71 with osteoporosis. The DNA was extracted by means of the use of the derived combined technique of the method fenol-sevag and inorganic, it stops later on to amplify the exclusive sequences of the end 3` of the gene RVD applying the reaction in Chain of the polymerase (PCR). The association among the genotypes of the gene receptor of the vitamin D and the normal established categories and osteoporosis, they were statistically significant, being the population in genetic equilibrium for the three polymorphism. The genotype BB was presented in the groups control and with osteopenia in percentages of 20.83% and 21.05%, while in the group with osteoporosis occupied 56.33%. In the same way, the genotype AA was presented in the group with osteopenia in 8.33%, followed by the group control 28.95%, and the group with osteoporosis with 50.70%. The genotype tt was in the groups control and with osteopenia in percentages of 0- 10.53%, and in the group with osteoporosis in 25.35%. The haplotype BBAA_{tt} turned out to be a factor of risk for the osteoporosis, with a relative risk estimated by Ott of 4.4; while the haplotype Bbaa_{TT} showed to be a protection factor. These discoveries allow establishing association in our population of these genotypes of the RVD gene with the osteoporosis, just as it has been reported in other countries.

Analysis of the Alcohol Dehydrogenase (ADH3) and the Serine Protease Inhibitor, Kazal type 1 (SPINK1) Genes in Alcohol-Related Pancreatitis. *K.G. Monaghan, M.A. Kilar.* Medical Genetics, Henry Ford Hospital, Detroit, MI.

Background and Objectives: Alcohol dehydrogenase (ADH3) polymorphisms and serine protease inhibitor, Kazal type 1 (SPINK1) mutations, particularly N34S, have been examined in pancreatitis patients, although no study has analyzed both genes in the same patient. Previous studies suggested a possible association between ADH3 or SPINK1 and alcohol induced pancreatitis. We hypothesize that ADH3 polymorphisms and SPINK1 mutations are associated with alcohol-induced pancreatitis. **Experimental Approaches:** Three groups were analyzed in this study: Patients with alcohol-induced pancreatitis (N = 45), non-alcohol associated pancreatitis with a positive family history of pancreatitis (N = 8) and non-alcohol associated pancreatitis with a negative family history of pancreatitis (N = 51). The ADH3 *1 and *2 polymorphisms were analyzed by restriction fragment length polymorphism (RFLP) analysis. SPINK1 exon 3 was analyzed by direct DNA sequencing. **Results:** The ADH3 *1 and *2 allele frequencies between Caucasian and African American patients and normal controls did not differ. SPINK1 mutations were only identified among patients with non-familial, non-alcohol induced pancreatitis. Six of fifty-one patients (11.8%) had at least one SPINK1 mutation, including N34S, P55S, IVS3+2TC, and a novel mutation, V46D. One individual was compound heterozygous for N34S and IVS3+2TC. **Conclusions:** ADH3 is not likely to be involved in alcohol-induced pancreatitis in Caucasians or African Americans. Our results are in agreement with previous findings, that SPINK1 mutations are associated with non-familial idiopathic pancreatitis. However, our results suggest that SPINK1 mutations are not likely involved in alcohol-induced pancreatitis among Caucasians or African Americans. SPINK1 mutation analysis may be beneficial in determining the etiology of the disease in patients affected with idiopathic pancreatitis. Further studies regarding the penetrance of SPINK1 mutations are needed prior offering testing to asymptomatic at-risk family members.

Multi-dimensional Clustering Analysis using pulsed/non-pulsed Neural Network algorithms to extract Gene SNP molecular epistasis from a multiplex genotype in Deep Vein Thrombosis Studies. *D. Ross¹, J. Perrett², A. Arellano¹, L. Bare¹, F. Rosendaal³, S. Petitti⁴, J. Sninsky¹.* 1) Celera Diagnostics, Alameda, CA; 2) DNN Industries, Chippenham, UK; 3) LUMC, Leiden, The Netherlands; 4) Kaiser Permanente DOR, Oakland CA.

Complex genetic diseases in humans are responsible for major clinical and diagnostic burden on society. Complex interactions of multiple genes may underlie complex disease, a concept known as epistasis. DVT was chosen as a test case because there are previously reported SNPs (Factor V Leiden (FVL) and FII G20210A) and environmental factors (e.g., contraceptives) that influence its occurrence. Widespread testing for FVL in DVT has been constrained for multiple reasons, including the low allele frequency and the moderate risk for the heterozygote. Two datasets totaling 1100 individuals (49% cases and 51% controls) with phenotype for DVT, age, contraceptive use and sex were genotyped with a multiplex assay of 25 different markers. Multi-dimensional clustering analysis (MCA), which integrates evolutionary programming, pulsed and non-pulsed neural networks in a true parallel processing architecture, was used to identify markers, and their association with the case population. Under MCA, all marker and phenotype combinations were evaluated simultaneously. A group of gene-marker-genotype combinations were significant, and extracted as a group. Among the gene/SNP interactions associated with clinical outcome are FVL, FII-G20210A, CBS-G919A and TFPI-C536T. The identified group of gene/SNP markers suggests molecular epistasis of genes involved in the coagulation pathway. The risks of FVL and FII-20210A were supported by simple association tests. ROC curves were used to determine the predictive value of the group of gene/SNP markers as a diagnostic. The SNP group is more powerful (AUC 0.70) than, for example, testing for FVL alone (AUC 0.63), or FVL plus FII G20210A alone (AUC 0.65). The MCA procedure has the advantages of evaluating all of the interactions simultaneously, construction of algorithmic model for predictive diagnostic value and the ability to detect interactions that are not always reveal by other statistical tests.

Frequency and nature of Angelman syndrome *UBE3A* mutations in Japan. S. Saitoh, K. Hosoki, K. Takano, A. Sudo. Dept Pediatrics, Hokkaido Univ Sch Medicine, Sapporo, Japan.

About 10% of Angelman syndrome (AS) cases are caused by a mutation of *UBE3A*. The *UBE3A* gene shows tissue-specific imprinting with only the maternally derived allele expressed in certain areas of the brain. More than 50 mutations of *UBE3A* have been described in AS, all that are either maternally inherited or have arisen *de novo*. We have investigated 5 patients from 3 families and 30 sporadic patients with AS phenotypes in Japan for whom the *SNURF-SNRPN* DNA methylation test revealed a normal methylation pattern, ruling out a large deletion, uniparental disomy or imprinting defects. PCR amplification of genomic DNA and direct sequencing of all coding exons and flanking sequences was performed. We identified potential mutations in all 5 patients of 3 families (100%), and for 6 of 30 sporadic patients (20%). These included IVS14-2AG, 668delA and 1632_1633CGGA (S349X) in familial patients, and 3089_3094del6, IVS15-3_3030del9, 1842CG (P419R) and 3093_3096del4 in 6 sporadic patients. The 3093_3096del4 mutation was found in 3 independent patients and indicates a mutation hot spot in Japanese, while the other mutations were only identified in single patients. Two mothers of familial cases were carriers for the mutations, while another did not have the mutation and was most likely to have germline mosaicism. We could investigate only two mothers of sporadic patients and neither were carriers, suggesting that the mutations arose *de novo*. In conclusion, *UBE3A* mutations are commonly identified in familial patients but in only 20% of sporadic patients. This mutation detection rate is consistent with previous studies, indicating that *UBE3A* mutations are not the major cause of AS in sporadic patients with normal DNA methylation. A genotype-phenotype correlation was not conclusive in our series of patients. Nonetheless, clinical severity was relatively mild in these patients when compared to patients with a 5 Mb deletion. The identification of germline mosaicism for *UBE3A* mutations in our series of patients and the low frequency of *UBE3A* mutations in AS further highlights the significance and difficulty of genetic counseling in patients with AS.

Variation in the CYP2C9 and SCN1A genes influences clinical use of the anti-epileptic drug phenytoin. *S. Tate, C. Depondt, S. Sisodiya, S. Schorge, S. Shorvon, J. Sander, N. Wood, D. Goldstein.* Dept Biol, Univ Col London, London, United Kingdom.

Phenytoin is an effective and inexpensive anti-epileptic drug (AED) less widely used in Europe now, partly because of concerns about adverse reactions (ADRs). As with many AEDs, a broad range of phenytoin doses are used, with the final "maintenance" dose normally determined by trial and error. Starting doses are variable and may be reduced on presentation of one or more adverse reactions or increased if seizures are not controlled. Phenytoin also has a narrow therapeutic index, and nonlinear kinetics mean that small increases can result in disproportionate increases in serum levels. Empirical calibration of dose therefore may entail significant clinical harm in terms of delayed efficacy or adverse reactions. For these reasons phenytoin is an excellent candidate for pharmacogenetic diagnostics. If gene variants could be identified that predict appropriate starting doses or that identify individuals more likely to suffer ADRs, phenytoin could be used more effectively and with fewer side effects. There are also well-defined candidate genes that could harbour variants that influence drug response. Phenytoin targets voltage-gated sodium channels in the brain, encoded by the SCN family of genes (including the SCN1A, implicated in Mendelian forms of epilepsy). It is a substrate of P-glycoprotein, an efflux pump expressed at the brain blood barrier encoded by ABCB1, and the major drug metabolising enzyme is CYP2C9. We therefore assessed how variation in the CYP2C9, SCN1A, and ABCB1 genes influences the maximum dose (in most cases equivalent to the maintenance dose) of phenytoin in 270 patients treated with phenytoin. We report here that polymorphisms in both the CYP2C9 and SCN1A genes show highly significant associations with maximum dose. This retrospective study implies that genetically defined subgroups of patients require, on average, maintenance doses ranging from 250 to 376 mg of phenytoin per day, and sets the stage for a prospective evaluation of how pharmacogenetic diagnostics can be used to improve the use of phenytoin.

Association of novel insertion in NPHS2 (Podocin) gene with End-Stage Renal Disease (ESRD) in African Americans. *J.A. Engeler Dusel*^{1,2}, *K.P. Burdon*³, *P.J. Hicks*³, *B.I. Freedman*^{1,2}, *D.W. Bowden*^{2,3}. 1) Nephrology; 2) Internal Medicine; 3) Biochemistry, Wake Forest Univ School of Med, Winston-Salem, NC.

Podocin, encoded by NPHS2 and mapped to 1q25-31, is an integral membrane protein exclusively expressed in glomerular podocytes. Mutations in NPHS2 have been shown to cause autosomal recessive nephrotic syndrome and have been associated with proteinuria in several populations. We identified moderate evidence for linkage to ESRD on chromosome 1q25.1 in the region of NPHS2 (LOD 1.62) in a genome-wide scan in 296 African American (AA) siblings concordant for non-diabetic (non-DM) ESRD making this gene a positional candidate. We sequenced all coding regions and 2kb of promoter sequence of NPHS2 in 96 unrelated AA non-DM ESRD cases and 96 healthy AA population-based controls. Non-DM ESRD subjects (predominantly hypertension-associated ESRD) had a meanSD age at ESRD onset of 45.814.9 years and mean body mass index of 26.38.2 kg/m². Fifty three polymorphisms were identified with minor allele frequencies ranging from 1% to 44%, including 4 insertion/deletions. Twenty four polymorphisms were located in the promoter region, 10 were exonic, 13 intronic and 6 in the 3UTR. None were previously described as disease causing mutations. Four were novel non-synonymous SNPs (P20L, A44E, A61V and A242V). A242V showed borderline association with a frequency of 0.032 in cases and 0.074 in controls (p-value=0.06). This SNP and three relatively common synonymous coding SNPs (T1023C, C1275G and A1479G) were genotyped in an additional 192 cases and 96 controls, however, none were significantly associated. An A insertion polymorphism in intron 3, 9 bp downstream of exon 3 and close to the splice site was detected in 6 cases and no controls in the original sequencing. Statistical analysis demonstrated single SNP association to ESRD (p-value=0.014; Fishers exact test). This variant is undergoing further evaluation in the larger sample. In conclusion, although coding variants do not appear to contribute significantly to ESRD in the general population, the intron 3 insertion may be associated with non-DM ESRD in African Americans and NPHS2 may contribute to the development of this disease.

A novel approach for genetic predisposition to diabetic nephropathy. *A.J. McKnight, K.A. Pettigrew, D.A. Savage, A.P. Maxwell.* Nephrology Research Group, Queen's University of Belfast, Northern Ireland.

Diabetic nephropathy is the leading cause of end stage renal failure in the western world. There is substantial epidemiological evidence supporting genetic predisposition, but the exact model of inheritance is unknown. A number of conflicting association results have been reported, reflecting several factors including the limited examination of candidate genes, variation in selection criteria, and relatively small sample sizes.

We recently completed the first genome-wide, association screen using pools of genomic DNA derived from accurately phenotyped individuals. Caucasian type 1 diabetic patients with (case) and without (controls) nephropathy were recruited from Ireland. We employed 6000 microsatellite markers indirectly distributed throughout the genome, resulting in a final map resolution of 0.7 cM (~590 kb). All putatively associated regions from our initial screen were confirmed in repeat pools and the top 50 markers individually genotyped to confirm the association.

Independently, we developed a unique candidate gene directory for diabetic nephropathy. This resource contains current information on >1000 genes reportedly involved in the pathophysiology of diabetic nephropathy. The data was extracted from a variety of sources and collated into a centralised, readily accessible, web-based repository with all information presented for query and download in several formats. There are currently 1068 genes in the database [June 2004].

By combining these two approaches (positional and functional) we have identified several genomic regions and prioritised candidate genes that may be associated with diabetic nephropathy. Candidate genes are screened for variants using WAVE (dHPLC) technology. Variants are confirmed by direct capillary sequencing and genotyped using TaqMan, Invader or Pyrosequencing technologies in our All Ireland case (n=300) control (n=363) collection. Initial analysis of several candidate genes has revealed association with nephropathy for a potentially functional promoter variant *VEGF*-460C>T ($p<0.001$).

Genome-wide association study of idiopathic Parkinson's disease using 27,000 microsatellite markers. W.

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Parkinson's disease (PD), one of the most common human neurodegenerative diseases, is a complex disorder with multiple genetic and environmental factors influencing disease risk. Although several causal genes for Mendelian inherited PD (alpha-synuclein, parkin, UCH-L1, DJ-1, NR4A2, and PINK1) have recently been identified, genetic factors that influence idiopathic PD as strongly as APOE-E4 influences Alzheimer disease have not yet been identified. To identify susceptibility genes for idiopathic PD, we performed a genome-wide association study using approximately 27,000 microsatellite markers arranged at intervals of approximately 100kb throughout the genome. For the initial screening, we performed the association study with pooled DNA from 124 patients with PD and 124 normal controls. We analyzed the PCR products with the GeneScanTM software, and compared the pattern of the PCR products of pooled DNA from patients with PD and controls by the PickPeak software. We have finished analyzing the approximately 27,000 markers distributed throughout all the chromosomes and found associations ($p < 0.05$) in approximately 8% of the markers. We are currently performing systematic and comprehensive second and third screenings on all of these candidate markers using other sets of pooled DNA to exclude false positive associations, and expect that 10-20 markers will show significant associations throughout all three screenings. We will subsequently confirm these significant associations using individual samples. Genes in linkage disequilibrium with these markers may be associated with the pathogenesis of PD.

Quantitative perturbations of p53 (Real-time PCR) in Endometriosis: Differences between American and Icelandic Cases. *JL. Simpson*^{1,2}, *J.T. Gylfason*³, *R.T. Geirsson*^{3,4}, *D. Dang*¹, *K. Benediktsdottir*⁴, *V. Petursdottir*⁴, *A. Poindexter*¹, *J. Buster*¹, *S. Carson*¹, *F.Z. Bischoff*¹. 1) Depts OB/GYN; 2) and Hum/Mol Genet. Baylor Col of Med, Houston TX; 3) Faculty of Medicine, Univ Iceland; 4) Depts OB/GYN and Pathology, Landspítali Univ Hospital, Iceland.

Endometriosis is a non-neoplastic disorder, but shares similarities with malignancy (i.e. local invasion and metastasis). An attractive model for the underlying genetic cause in endometriosis is the multi-step mutation pathway. Using FISH techniques, we have shown that perturbations of chromosome 17 and the p53 locus occur frequently in severe/late stage endometriosis. Although reliable, FISH enumeration of signals is subject to observer skill and bias. We now report the use of quantitative real-time PCR to assess aberrations involving p53 copy number in eutopic endometrial and endometriotic tissue specimens. In addition, we compare American and Icelandic cases. **METHODS:** For American cases, fresh tissue specimens (n=21) and peripheral blood samples were obtained from women with advanced stage disease. Controls consisted of peripheral blood from 28 unaffected women undergoing tubal ligation. For Icelandic cases, paraffin embedded endometriotic and matched eutopic endometrial tissue (n=19) were obtained from women with advanced stage disease. DNA from tissue specimens was extracted and real-time quantitative PCR performed to quantify copies of the p53 gene compared to the ubiquitous GAPDH control locus. **RESULTS:** An expected normalized mean of 1.003 was observed for the p53 locus among control cases. Among samples collected in the USA, significant p53 gain (n=13) or loss (n=4) was observed in all but 4 of 21 cases compared to GAPDH. Surprisingly, we failed to observe either gain or loss of p53 in Icelandic patients (n=19). **CONCLUSIONS:** Our results support the role of non-random somatic alterations involving the p53 locus in the pathogenesis of most cases of late/severe stage endometriosis. Differences between Icelandic and American subjects could have implications for generalizability of population based genome wide approaches.

Large-scale case control study for bronchial asthma. *M. Tamari¹, T. Hirota¹, A. Matsuda¹, M. Akahoshi¹, K. Obara¹, N. Takahashi¹, M. Shimizu¹, S. Doi³, A. Miyatake⁴, K. Fujita⁵, T. Enomoto⁶, H. Saito⁷, Y. Suzuki⁸, Y. Nakamura⁹, T. Shirakawa^{1,2}.* 1) Lab for Genetics of Allergy, RIKEN SNP Research Ctr, Yokohama, Kanagawa, Japan; 2) Dept. of Health Promotion and Human Behavior, Kyoto University, Kyoto, Japan; 3) Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Osaka, Japan; 4) Miyatake Asthma Clinic, Osaka, Japan; 5) School of Human Nursing, The University of Shiga Prefecture, Shiga, Japan; 6) Japanese Red Cross Society, Wakayama Medical Center, Wakayama, Japan; 7) National Research Institute for Child Health & Development, Tokyo, Japan; 8) Department of Medical Genetics, Tohoku University School of Medicine, Sendai, Japan; 9) The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

Bronchial asthma is a complex disorder caused by combination of genetic and environmental factors. SNPs (Single nucleotide polymorphisms) are the most common form and are thought to be useful markers to identify genes susceptible human common disease. Systematic analysis to search an association between SNPs genotype and disease phenotype is expected to disclose a gene(s) related to complex diseases. To clarify the genetic factor implicated in the etiology of bronchial asthma, we have conducted genome-wide association studies with 86,100 randomly selected gene-based SNPs using the high-throughput multiplex PCR-Invader assay method. After first screening with 86,100 SNPs (94 asthmatic patients vs. 658 control samples), we have focused on 2026 SNPs revealed positive association ($p < 0.01$). Next, we have conducted association study in the second sample (470 childhood asthma, 470 adult asthma and 658 controls). Finally, 17 SNPs were significantly associated with asthma susceptibility ($p < 0.001$). A SNP#A was significantly associated with asthmatics ($p = 0.0000074$). The gene containing SNP#A express in bronchus, testis and lung tissues and lies on chromosome 3p21, a locus linked to asthma susceptibility. Using real-time quantitative RT-PCR, transient transfection reporter gene assays, EMSA, mRNA stability assays and immuno-histochemistry, functional analyses of genes containing these SNPs are on going.

Sequence Variants of the *DRD4* Gene in Autism; Further Evidence that Rare *DRD4* 7R Haplotypes are ADHD Specific. *D.L. Grady*¹, *A. Harxhi*², *M. Smith*², *P. Flodman*², *M.A. Spence*², *J.M. Swanson*², *R. Moyzis*^{1,2}. 1) Dept Biological Chemistry, Univ California Col Medicine, Irvine, CA; 2) Department of Pediatrics, University of California, Irvine, Medical Center, Orange, California, 92868, USA.

A high prevalence of rare dopamine receptor D4 (*DRD4*) alleles in children diagnosed with attention-deficit hyperactivity disorder (ADHD) has been reported (Grady DL, Chi H-C, Ding Y-C, Smith M, Wang E, Schuck S, et al. 2003, *Molecular Psychiatry* 8:536-545). In this prior study, extensive haplotype data of the *DRD4* locus was used to suggest that population stratification was not the explanation for the high prevalence of rare alleles. However, the population sample used as a control was obtained from diverse geographic locations, and hence specific local haplotype variants could have contributed to these observations. In order to test this possibility, DNA resequencing/haplotyping was conducted on 136 *DRD4* alleles obtained from autism probands, collected from the same geographic population as the prior ADHD probands (Orange County, CA). A number of studies have suggested that the susceptibility genes underlying these two disorders might partially overlap. Rare *DRD4* variants were not uncovered in this autism sample beyond that expected by chance. These results suggest strongly that the high prevalence of rare *DRD4* alleles in ADHD probands is due to ascertainment of the sample by diagnosis of ADHD.

Analysis of genetic variation and expression of the dysbindin1 gene in schizophrenia. *D.B. Wildenauer^{1,2}, S.G. Schwab^{2,3}, S. Mondabon⁴, M. Knapp⁵, M. Albus⁶, M. Borrmann-Hassenbach⁶, W. Maier⁴, A. Schmidt⁷, M. Hoehe⁸, B. Timmermann⁸, R. Reinhard⁸.* 1) CCRN, Claremont, WA, Australia; 2) School of Psychiatry, University of Western Australia, Perth, Australia; 3) WAIMR, Perth, Australia; 4) Dept of Psychiatry, University of Bonn, Germany; 5) Dept of Medical Statistics, University of Bonn, Germany; 6) Mental State Hospital, Haar, Germany; 7) ZI, Mannheim, Germany; 8) MPI Molecular Genetics, Berlin-Dahlem, Germany.

We reported recently association of single nucleotide polymorphisms within the dysbindin1 gene with schizophrenia in two independent family samples (Schwab et al, 2003, *Am J Hum Genet* 72:185-190) one sample comprising 79 families with at least two affected siblings and both parents available for genotyping and the second sample comprising 128 trio families (affected offspring with parents). None of the reported markers are located in exons or are known to cause functional or structural changes in the gene products. Our goal was to find the susceptibility alleles involved in development of schizophrenia. DNA sequence analysis has been used to analyze the genomic region for further variants. 96 patients with schizophrenia from our family samples have been included in the analysis. 79 DNA variants have been identified by sequence analysis, 29 having a minor allele frequency of 0.10 in the analyzed sample. So far, no exonic variation has been detected in the investigated region. In addition, we performed expression analysis using real time technology for relative quantification of RNA levels. 62 RNA samples isolated from immortalized lymphoblast cell lines from the patients of our family samples as well as RNA samples isolated from post mortem brain of 10 schizophrenic patients and 10 controls were available. No differences in RNA levels of dysbindin1 have been identified in post mortem brain tissue. However, preliminary results indicate statistical significant differences in the expression levels of dysbindin1 in lymphoblasts of our patients, if genotype information was used as a co-variant in the statistical analysis. The failure to detect exonic variants as well as the differences in gene expression suggests involvement of variants affecting transcription rate.

Lack of Association and Linkage of the Serotonin-2A Receptor Gene to Autism Spectrum Disorders. A.

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Hyperserotonemia is one of the most consistent biochemical findings in Autism Spectrum Disorders (ASDs). It has recently been reported that autistic individuals with hyperserotonemia have decreased serotonin 2A (5HT2A) receptor binding. Antagonists of the 5HT2A receptor have been shown to reduce some symptoms of autism. These findings suggest that, based on its function, the HTR2A gene is a good candidate gene for autism susceptibility. In addition, two recent genome scans showed evidence for linkage of autism susceptibility to chromosome 13q, the location of the HTR2A gene, providing evidence for it as a positional candidate locus. We genotyped a SNP in the promoter region HTR2A (1438A/G) in 190 multiplex families, with 388 affected individuals. Of these, 138 families were from AGRE. The comparison group consisted of DNAs from 116 placentae, 138 controls and 334 random anonymous newborn bloodspots obtained from the Ontario Ministry of Health Newborn Screening Program and previously used for PKU testing. Of the multiplex families, 119 had at least two affected males, 59 had at least one affected male and one affected female, and the remainder had only affected females. The Family-Based Association Test (FBAT) did not show any preferential transmission of specific alleles in any of the family groups. Case-control comparisons showed that the genotype and allele frequencies in affected individuals did not differ statistically from sex-matched controls. Genotype-phenotype associations using the ADI-R data showed that the mean ADI-R scores for the 3 domains of autism, age-of-onset, and age at first phrase did not differ in individuals with different HTR2A genotypes. These results do not support a major role for the HTR2A gene in susceptibility to ASD, confirming findings by Veenstra-VanderWeele et al (2002) (*Am J Med Genet* 114:277-283). Supported by research grants from CIHR and OMHF to JJAH.

***CNTF* gene polymorphisms are associated with muscle strength variation in older adults.** D.E. Arking¹, T. Li², J. Walston², D. Fallin³, B. Beamer², Q.L. Xue², L. Fried², A. Chakravarti¹. 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Johns Hopkins University Older Americans Independence Center, Baltimore, MD; 3) Department of Epidemiology, Johns Hopkins University Bloomberg School of Public Health.

Skeletal muscle strength declines with age, and plays an important role in the development of disability and frailty. The etiology of strength declines is multifactorial, including decreasing levels of activity, increased inflammation and disease burden, declines in nerve/muscle synapses, and genetic variation. Ciliary Neurotrophic Factor (*CNTF*) is expressed in motor neurons where it protects the cells from apoptosis. We hypothesized that variation in genes that influence skeletal muscle mass maintenance will contribute to differences in muscle strength and disability. We tested this in the Womens Health and Aging 1 and 2 Cohorts of community dwelling older women, by genotyping 359 women between age 70-79 at 8 single nucleotide polymorphisms (SNPs) in and around the *CNTF* gene. We performed linear regression analyses, adjusting for age, BMI, osteoarthritis, and race, and identified significant associations between homozygosity for rs1800169 A allele and lower grip strength (-4.152.80, p0.004), and an additive effect of the rs2510559 T allele on knee strength (-1.811.27, p0.005). The rs1800169 A allele has been previously shown to be a functional variant, resulting in a complete absence of *CNTF* protein in homozygous individuals. These findings suggest that *CNTF* gene variation may play an important role in the loss of muscle strength, which in turn influences disability and frailty in older adults. These findings also provide evidence of physiologic pathways that may warrant future intervention studies in frail older adults.

Polymorphisms in the *Phospholipase A2 Group 2D (PLA2G2D)* gene on chromosome 1 are associated with asthma in U.S. Hispanics. *M.J. Basehore¹, E.R. Bleecker¹, L.A. Lange¹, M.S. Harkins², E.J. Ampleford¹, W.C. Moore¹, D.A. Meyers¹, T.D. Howard¹.* 1) Wake Forest Univ Sch Med, Ctr for Human Genomics, Winston-Salem, NC; 2) University of New Mexico, Dept. of Medicine, Albuquerque, NM.

Asthma is a common respiratory disease caused by airways inflammation and bronchial hyperresponsiveness (BHR) that results in variable airway obstruction and clinical symptoms. BHR represents an increased bronchoconstrictor response found in asthma and is measured by the methacholine concentration that causes a 20% decline in lung function (PC_{20} FEV₁). Previously, we have shown evidence for linkage to 1p32-p34 for asthma (LOD=2.92; Xu et. al, 2001) in 40 Hispanic families, as part of the Collaborative Study for the Genetics of Asthma. Several candidate genes reside within this region of linkage, including three of the 10 human phospholipase A2 genes. These three genes, *PLA2G2A*, *PLA2G5*, and *PLA2G2D*, are located in tandem on the chromosome. Association with asthma or a related phenotype was evaluated across the three genes using publicly available polymorphisms. The individuals studied were African-Americans (168 asthmatics; 269 controls), U.S. Caucasians (233 asthmatics; 245 controls), and U.S. Hispanics (116 asthmatics; 130 controls) from a case-control asthma population. Genetic analysis revealed the association of a *PLA2G2D* coding SNP (S80G) with PC_{20} in the Hispanic asthmatics ($p=0.004$). Subsequently, ten additional SNPs in *PLA2G2D* were evaluated for association, and two were also found to be associated with PC_{20} in the Hispanic asthmatics ($0.002 \leq p \leq 0.036$). There was a strong haplotype effect in this population across the *PLA2G2D* locus, and association of haplotypes with both asthma ($p=0.003$) and PC_{20} ($0.0006 \leq p \leq 0.028$) was observed. These data suggest that either single SNPs or haplotypes within *PLA2G2D* may influence the susceptibility and severity of asthma as well as the severity of airway constriction.

Two-loci association of insulin secretion in obese children. *P. BOILEAU¹, S. LE FUR¹, D. FRADIN¹, J.M. BAZIOMO², C. LE STUNFF¹, P. BOUGNERES¹.* 1) Dpt of Pediatric Endocrinology & U561 INSERM, Hôpital Saint-Vincent de Paul, Paris, France; 2) Dpt of Pediatrics, Hôpital de Ouagadougou, Burkina Faso.

The KIR6.2 gene encodes subunits of the KATP channel which regulates insulin exocytosis from b cells. The highly polymorphic INS VNTR located in the insulin gene promoter is associated with changes in transcriptional activity of the insulin gene. E23K, a common variant of KIR6.2 in Caucasians, leads to decreased insulin release in vitro, but its effects on insulin secretion are less easy to demonstrate in vivo. In 570 obese children, E23K and INS VNTR alleles showed only marginal effects on insulin secretion when each of the loci was examined individually. But when the two loci were combined for analysis, we found that obese children with KIR6.2 EE / VNTR I/I genotypes secreted more insulin than all other genotype combinations. This difference was important in boys (insulinogenic index: 97.14 in E/E & I/I; 51.6 in KK & I/III or III/III, $p < 0.005$; 74.4 in other genotypes $P=0.015$). Two West African populations were found to lack the E23K variant and have different VNTR alleles: the genotypic effects observed in Caucasians were therefore absent. These observations indicate the importance of the joint analysis of candidate genes in association studies of complex traits and illustrate the heterogeneity of genetic factors in different human groups.

Agouti-related protein (AGRP) promoter variant is associated with leanness and decreased risk for diabetes in West Africans. *C. Bonilla¹, R.K. Panguluri¹, L. Taliaferro-Smith¹, G. Argyropoulos², G. Chen¹, A.A. Adeyemo¹, R.A. Kittles¹, C. Rotimi¹, AADM study group.* 1) National Human Genome Center at Howard University, Washington, DC; 2) Pennington Biomedical Research Center, Baton Rouge, LA.

The role of the central melanocortin system in the development of obesity has been extensively studied. Single nucleotide polymorphisms (SNPs) within several candidate genes have been associated with food intake and obesity-related phenotypes however few of these associations have been replicated. SNPs in the Agouti-related protein (AGRP) gene coding (Ala67Thr, 199G/A) and promoter (-38C/T) regions have been reported to be associated with body mass index (BMI), fat mass (FM), and percent body fat, in populations of European and African descent. Given that AGRP is a natural antagonist of melanocortin receptors 3 and 4, which inhibit food intake, variants such as the -38C/T that alter promoter activity and affinity for transcription factors are likely to influence body composition and energy balance. In this study, we evaluated the association between the functional AGRP -38C/T promoter SNP and weight-related traits, namely BMI, FM and fat free mass (FFM), as well as on diabetes status in a well characterized population of 538 West Africans from Ghana and Nigeria. We report that women carrying two copies of the variant T allele had significantly lower BMI (OR=0.47; 95% CI, 0.26-0.89). Also, men with at least one copy of the variant T allele were over two times less likely to be diabetic than other men (OR=0.45; 95% CI, 0.22-0.91). Our results replicate previous findings and strongly implicate the AGRP -38C/T SNP in the regulation of body weight in West Africans. Whether the association with Type 2 diabetes is a consequence of the energetic imbalance caused by an increase in food intake or is a result of the independent action of AGRP on insulin secretion needs further investigation.

Pharmacogenetic Differences in Response to Bronchodilators Between Puerto Rican and Mexican Asthmatics. *E. Burchard*^{1,2}, *S. Choudhry*^{1,2}, *N. Ung*^{1,2}, *P.C. Avila*¹, *E. Ziv*¹, *S. Nazario*³, *J. Casal*³, *A. Torres*³, *K. Salari*^{1,2}, *J.R. Rodriguez-Santana*⁴, *J. Salas*⁵, *M. Selman*⁵, *R. Chapela*⁵, *D. Sheppard*^{1,2}, *S.T. Weiss*⁶, *J.G. Ford*⁷, *H.A. Boushey*¹, *J.M. Drazen*⁶, *W. Rodriguez-Cintron*³, *E.K. Silverman*⁶, from the *Genetics of Asthma in Latino Americans (GALA) Study*. 1) University of California, San Francisco, SF, CA; 2) Lung Biology Center, San Francisco General Hospital, SF, CA; 3) San Juan VAMC, University of Puerto Rico School of Medicine, San Juan, PR; 4) Pediatric Pulmonary Program of San Juan, San Juan, PR; 5) Instituto Nacional de Enfermedades Respiratorias (INER), Mexico City, MX; 6) Brigham and Womens Hospital, Boston, MA; 7) The Harlem Lung Center, Harlem Hospital and Columbia University, New York, NY.

Background: In the U.S., Puerto Ricans and Mexicans have the highest and lowest asthma prevalence, morbidity and mortality, respectively. Whether ethnic-specific differences in the response to drug treatment contribute to differences in outcome is unclear. Albuterol is the most commonly prescribed asthma medication worldwide. Genetic variants at the beta2 adrenergic receptor (2AR) may modify asthma severity and albuterol responsiveness. **Methods:** We used both family-based and cross-sectional analysis to test for association between 2AR SNPs and asthma severity and bronchodilator response in 684 Puerto Rican and Mexican families. **Results:** Among Puerto Ricans, the Arg16 allele was associated with greater bronchodilator response using both cross-sectional and family-based tests ($p = 0.01-0.00001$). In addition, we found a strong interaction of baseline Forced Expiratory Volume or FEV1 with the Arg16Gly polymorphism in predicting bronchodilator response. Among Puerto Rican asthmatics with FEV1 < 80% of predicted, but not in those with FEV1 >80%, there was a very strong association between this genotype and bronchodilator response. In contrast, there were only marginal associations between b2AR genotypes and drug responsiveness among Mexican asthmatics and these associations differed from those found in Puerto Ricans. **Conclusions:** Ethnic-specific pharmacogenetic differences in response to bronchodilator exist between Puerto Rican and Mexican asthmatics.

C-Reactive Protein (CRP) Haplotypes are Associated With Cardiovascular Risk Factors but not Insulin

Resistance: the IRAS Family Study. *I. Chen^{1,2}, K.D. Taylor^{1,2}, X. Guo^{1,2}, J. Cui¹, M.F. Saad², L.E. Wagenknecht³, S.M. Haffner⁴, J.I. Rotter^{1,2}.* 1) Cedars-Sinai, Los Angeles, CA; 2) UCLA, Los Angeles, CA; 3) Wake Forest University School of Medicine, Winston-Salem, NC; 4) University of Texas Health Center, San Antonio, TX.

CRP levels, an inflammatory biomarker, have been associated with several components of the metabolic syndrome, as well as incident diabetes and cardiovascular disease (CVD). Associations have been reported between variation in the CRP gene and plasma CRP levels. We examined the primary metabolic manifestations of variation in the CRP gene in a family cohort of Hispanic Americans (820 studied in San Luis Valley, CO and San Antonio, TX), and of African Americans (336 studied in LA, CA) in the IRAS Family Study. CRP (g/ml) and insulin (IU/ml) levels were determined by ELISA; fasting glucose (FG) (mg/dl) and lipids (mg/dl) by enzymatic colorimetry; Insulin sensitivity (IS) by the minimal model analysis of the frequently sampled intravenous glucose tolerance (FSIGT) test. Using the TaqMan MGB assay, we genotyped 5 SNPs in the CRP gene (rs1800947, rs2808630, rs2808631, rs3093062, rs3093066). Haplotypes were reconstructed using Simwalk2 and associations were tested using the General Estimating Equations (GEE) methods. We observed 6 haplotypes (H) with frequency 2% in founders (H1-H6). H1 was associated with a decreased FG (93.2 vs 96.5, $p=.001$), H3 with decreased TG (78.1 vs 122.4, $p=.023$), increased HDL-C (47.0 vs 43.8, $p=.016$), decreased TG/HDL-C ratio (1.90 vs 3.39, $p=.016$), and H4 with elevated CRP level (4.52 vs 3.14, $p=.011$) and higher FG (96.1 vs 93.6), but no association with IS. In summary, CRP gene haplotypes are associated, independently from IS, with 3 distinct CVD risk factors: CRP, FG, TG and HDL-C concentrations; suggesting that the role of inflammatory processes in diabetes and CVD associated with CRP may well be distinct from the role of insulin resistance related mechanisms.

Environment Dependent Association Between CD14 Gene and IgE Levels in Mexican and Puerto Rican Asthmatics. S. Choudhry^{1,2}, P.C. Avila¹, N. Ung^{1,2}, S. Nazario³, J. Casal³, A. Torres³, E. Ziv¹, J.R. Rodriguez-Santana⁴, J.K. Fagan⁵, J. Salas⁶, M. Selman⁶, R. Chapela⁶, D. Sheppard^{1,2}, H.A. Boushey¹, S.T. Weiss⁷, J.G. Ford⁵, J.M. Drazen⁷, W. Rodriguez-Cintron³, E.K. Silverman⁷, E.G. Burchard^{1,2}, from the *Genetics of Asthma in Latino Americans (GALA) Study*. 1) University of California, San Francisco, SF, CA; 2) Lung Biology Center, San Francisco General Hospital, SF, CA; 3) San Juan VAMC, University of Puerto Rico School of Medicine, San Juan, PR; 4) Pediatric Pulmonary Program of San Juan, San Juan, PR; 5) The Harlem Lung Center, Harlem Hospital and Columbia University, New York, NY; 6) Instituto Nacional de Enfermedades Respiratorias (INER), Mexico City, MX; 7) Brigham and Womens Hospital, Boston, MA.

Background: A genetic variant in the CD14 gene promoter, C-159T, has been associated with IgE and asthma in some studies, but not in others. Inconsistent results may reflect differences in environmental exposures, genetic heterogeneity, random error, or population stratification in case-control and cross-sectional studies leading to spurious associations. Family-based analyses are typically robust against the effects of population stratification. **Methods:** Using both family-based and cross-sectional designs, we analyzed the relationship between the CD14 C-159T genotypes and asthma-related phenotypes in Puerto Rican (n=393) and Mexican (n=274) asthmatics. In addition, we analyzed gene-by-environment interactions. **Results:** We found a significant association between the CD14 C-159T genotypes and log IgE in the presence of environmental tobacco smoke (ETS) exposure among Mexican and Puerto Rican asthmatics using both family-based (p=0.009 & 0.04, respectively) and cross-sectional analyses (p=0.07 & 0.00008, respectively). There were no significant associations between CD14 and IgE when analyses were performed among subjects who denied ETS exposure or when data were stratified by age, gender, or allergies to animals. **Conclusions:** The CD14 C-159T genotypes interact with tobacco smoke to modify IgE levels in asthmatics. Our data are consistent with multiple factors influencing asthma-related phenotypes including genetic and environmental.

Heme oxygenase-1 gene is associated with the rate of decline of lung function in smokers. *J-Q. He¹, P.D. Paré¹, J.E. Connett², N.R. Anthonisen³, A.J. Sandford¹*. 1) James Hogg iCAPTURE Center, University of British Columbia, Vancouver, Canada; 2) School of Public Health, University of Minnesota; 3) Faculty of Medicine, University of Manitoba.

Heme oxygenase-1 (HO-1) confers protection against oxidants in the lung. A (GT)_n repeats in the 5'-flanking region shows length polymorphism, and longer (GT)_n repeats were associated with lower transcriptional activity and pulmonary emphysema in Japanese individuals. However, we did not find association with rate of decline of lung function in whites. The aim of this study is to explore association of single nucleotide polymorphisms (SNPs) in the *HO-1* with rate of decline of lung function in smokers in the Lung Health Study (LHS). Five tag-SNPs (149G/A, 2790A/T, 3303G/C, 9531G/A and 15382A/G) in *HO-1* were selected from SeattleSNPs by linkage disequilibrium (LD) selection, which can select minimum number of SNPs to resolve the most common haplotypes. We selected 582 non-Hispanic whites with the fastest (n=278, FEV₁% predicted = -153.7 2.6ml/year) and with the slowest (n=304, FEV₁% predicted = 14.9 2.6ml/year) decline of FEV₁% predicted from among smokers followed for 5 years in the LHS. The associations were analyzed by logistic regression to adjust for potential confounding factors. The 9531G/A SNP, which is located at intron 3 but in perfect linkage with a SNP (2007G/A) in the promoter, was associated with the rate of decline of lung function. The frequencies of homozygous AA were 25.5% and 17.9% in smokers with fast decline and no decline of lung function respectively (unadjusted OR = 1.6, 95%CI = 1.1-2.3, p = 0.028). After adjusting for confounding factors such as age, sex, center, cigarette smoking and baseline of lung function, the adjusted OR = 1.8, 95%CI = 1.2-2.8, p = 0.006). This association was modified by methacholine responsiveness. The 9531G/A SNP is in LD with (GT)_n repeats with D' = 0.77 for longer (GT)_n allele (repeats 33). There were no associations between the other four SNPs and haplotypes with the rate decline of lung function. Further study of functional implications of 9531G/A and its relationship with (GT)_n repeats are warranted. Supported by the LHS and the CIHR.

A multilocus investigation of the dopaminergic pathway in Autism Spectrum Disorders. *J.A. Hettinger^{1, 3}, X. Liu^{1, 2, 3}, D.H. La^{1, 3}, J.J.A. Holden^{1, 2, 3}, ASD-CARC⁴.* 1) Department of Physiology, Queen's University, Kingston, Ontario, Canada; 2) Department of Psychiatry, Queen's University, Kingston, Ontario, Canada; 3) Autism Research Program, Ongwanada Resource Centre, Kingston, Ontario; 4) www.autismresearch.ca.

The identification of culprit genes for Autism Spectrum Disorders (ASDs) has proven elusive with conflicting findings reported on many candidate genes, mainly because of extensive phenotypic and genetic heterogeneity and limitations to the detection of genes of lesser or moderate effect. To overcome this, in our study of the dopaminergic system in ASD we examined family-based transmission (FBAT) of markers from genes involved in the synthesis, function, and degradation of dopamine. Findings implicating the dopaminergic system in a subgroup of individuals with ASD include decreased dopaminergic activity in the prefrontal cortex of autistic individuals and decreased levels of dopamine -hydroxylase (DBH) activity in affected individuals. In addition, we previously reported a possible maternal effect in which mothers in families with two or more affected sons had a higher frequency of the low activity DBH allele. We have now examined 22 polymorphisms in the PAH, TH, AADC, DBH, PNMT, and DAT genes in 132 multiplex (MPX- two or more affected individuals) families from the Autism Genetics Resource Exchange. Haplotype-FBAT was performed examining transmission of haplotypes. While single marker and gene haplotype transmissions were not significant on their own, there was over-transmission ($P=0.00076$) of 5 markers in the AADC (4bp Ins/Del, 826), DBH (-1021) and DAT (352, VNTR) genes. These findings suggest that a multilocus approach may permit the elucidation of the underlying pathways involved in the etiologies of ASDs. Further investigation, incorporating additional genes from the dopaminergic system and more extensive marker coverage, in conjunction with alternative analyses including recursive partitioning and ordered-subset analysis, are being used to determine the role of the dopaminergic pathway in ASD. Supported by a research studentship from OMHF to JAH and research grants from CIHR and OMHF to JJAH.

The genetic study of age at natural menopause: a familial aggregation and genetic association study. *Y. Hsu¹, H. Terwedow¹, Y. Feng¹, Y. Zhang¹, X. Xu^{1,2}.* 1) Prog Population Genetics, Harvard Univ, Boston, MA; 2) Anhui Medical Univ. Institute of Medicine, Anhui, China.

The purpose of this study was to explore the familial aggregation of age at natural menopause and the association between genetic variation in cyclooxygenase 2 lipoxygenase (ALOX12) and the age at natural menopause in a Chinese population. We initiated a community-based cross-sectional study among residents in Anhui Province, China since 2001. A total of 3304 women between 25-65 years of age from 1843 families were recruited. A standardized questionnaire was used to collect Subject's menstrual status, reproductive history, disease history, life style and dietary style. Intra sib pairs correlation coefficients of age at natural menopause, age at first menstruation and the duration (years) of menstruation were calculated. Regression model was used to test the family aggregation. A missense SNP (rs434473: A -> G; Asn322Ser) in ALOX12 gene was genotyped in women with menopause. There was 1245 (37.68%) women reported menopause naturally. The average age of menopause is 46.6 years old. The correlation coefficient (after adjusted current age) of age at natural menopause, age at first menstruation and the duration (years) of menstruation among siblings were 0.19(p<0.0001), 0.14(p=0.018) and 0.16 (p=0.007), respectively. If one of the siblings is menopause, after adjusted possible confounders, the other sibling has a significantly higher chance of being menopause. The adjusted OR was 8.89(p<0.0001). The age of menopause in women with GG genotype and GA genotype of SNP in ALOX12 gene were significantly older than the menopausal age in women with AA genotype. The adjusted regression coefficient is 2.39(p=0.006) and 1.67(p=0.033), respectively. Age at natural menopause, age at first menstruation and the duration (years) of menstruation were significantly associated among siblings. Genetic variation in ALOX15 gene is associated with age of natural menopause. Our findings suggest that genetic influences may play an important role in age of natural menopause.

Preliminary exploration of the Serotonin HTR1B gene and perfectionism as a trait in Attention Deficit

Hyperactivity Disorder. *A. Ickowicz¹, Y. Feng², K. Wigg², J. Quist³, T. Pathare¹, J. Crosbie¹, W. Roberts¹, M. Malone¹, R. Schachar¹, R. Tannock¹, J. Kennedy³, C.L. Barr^{1,2}.* 1) The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Cell & Molecular Division, Toronto Western Research Institute, University Health Network, Toronto; 3) Neurogenetics Section, Centre for Addiction & Mental Health, Toronto.

Serotonin [5-hydroxytryptamine (5HT)] plays an essential role in cognition, locomotor activity and the regulation of sleep, pain, mood, and aggression. Serotonin elicits its effects through a large family of receptors (HTR1-7). Polymorphisms of the HTR1B gene have been implicated in a variety of psychiatric disorders including Obsessive Compulsive Disorder (OCD) and Attention Deficit Hyperactivity Disorder (ADHD). Recent reports indicate that in clinical samples approximately 10% of ADHD subjects may experience co-morbid Obsessive Compulsive Behaviours (OCB) and increased perfectionism. The objective of this study was to investigate the association of obsessive behaviours, perfectionism and the HTR1B gene in a sample of 186 families with an ADHD proband. Six single nucleotide polymorphisms (SNPs) of the HTR1B receptor gene were genotyped using standard methods. In our sample, the subset of probands with confirmed ADHD diagnosis and OCB comorbidity was too small to enable a categorical analysis. However using a quantitative approach we observed significant evidence for an association of the perfectionism trait, as measured by the sub-scale E of the Conners Teacher Rating Scale (CTRS) and Conners Parent Rating Scale (CPRS) with one of the haplotypes generated by HBAT analysis (p values 0.040 and 0.054 respectively). Single marker analysis of the perfectionism trait performed using the FBAT program was not significant. Although these results are preliminary and should be interpreted with caution, they point in the direction of an association of the HTR1B gene and perfectionism as a quantitative trait in families with ADHD.

The molecular basis for *MPZ* truncating mutations that defines severity of inherited peripheral neuropathies. M.

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Mutations in the *MPZ* gene cause dominantly inherited peripheral neuropathies with varying severity from Charcot-Marie-Tooth (CMT) disease (relatively mild alleles) to Dejerine-Sottas Neuropathy (DSN)/congenital hypomyelinating neuropathy (CHN) (severe alleles). A subset of *MPZ* mutations includes nonsense and frameshift alterations that result in premature termination codons (PTCs). We previously demonstrated that PTCs in the internal exons trigger the nonsense mediated decay (NMD) pathway, leading to destabilization of mutant *MPZ* mRNA. These alterations are exclusively associated with the CMT phenotype. In contrast, most mutations associated with a more severe DSN/CHN phenotype result in PTCs within the terminal or penultimate exons, and thus are undetectable by NMD and are stably translated into mutant proteins with a potential dominant-negative activity. However, a subset of PTC mutations at the 3' end of *MPZ* escapes the NMD pathway, yet causes a mild CMT phenotype. To delineate the molecular basis for phenotypic expression of *MPZ* truncating mutations, we examined functional properties of *MPZ* proteins that escaped NMD. We found that the frameshift mutations that disrupt the transmembrane domain lead to an intracellular accumulation of mutant proteins, mainly within the endoplasmic reticulum. This is accompanied by the identification of a higher number of apoptotic cells. Our data support the view that late frameshift mutations with a conserved transmembrane domain have a minor toxic effect and thus function as loss-of-function alleles, whereas a subset of truncated *MPZ* proteins acts as dominant negative, interfering with trafficking of wild-type *MPZ* to the cell surface, leading to a more severe form of peripheral neuropathy. These findings suggest that, when PTCs escape NMD, functional properties of mutant proteins may define the resulting phenotype in *MPZ*-mutation-causing peripheral neuropathies.

A positive association between adrenoceptor 1a and BMI in schizophrenic patients with exposure to antipsychotics. *T. Lan*^{1, 2, 5, 6}, *H. Hsieh*^{1, 5}, *Y. Liu*³, *F. Chu*³, *H. Sun*², *K. Chang*^{2, 3}, *H. Chiu*^{4, 5}. 1) Inst Genetics, Natl Yang-Ming Univ, Taipei, Taiwan; 2) Dept Adult Psychiatry, Tao-Yuan Psychiatric Center, Tao-Yuan, Taiwan; 3) Tao-Yuan Hospital, Tao-Yuan, Taiwan; 4) Section Psychiatry, Natl Yang-Ming Univ, Taipei, Taiwan; 5) Yu-Li Hospital, Hualien, Taiwan; 6) Inst Genetics, Tzu-Chi Univ, Hualien, Taiwan.

Overweight and obesity have been identified as a severe epidemic among schizophrenic patients, especially compared to the general population (obesity: 5% vs. 17.4% in male, 7.9% vs. 27.9% in female in Taiwan). We recruited 213 schizophrenic patients treated with antipsychotics for more than 6 months with their consent form completed in this study. The body mass index (BMI) was continuously recorded per month for 6 months as the major outcome. We genotyped several candidate genes related to metabolism or obesity, including 5HT2A, ADRA1A, ADRA2A promotor, and ADRB3 for each participant. After adjusting for age, sex, and dosage effect of the target allele from 5HT2A, ADRA2A promotor, and ADRB3 genes, a statistically significant association under GEE distribution ($p=0.013$) between BMI and the genotypes of ADRA1A was found in these schizophrenic patients. The Hardy-Weinberg equilibrium of these candidate genes were tested to be held in our samples. There is also no significant difference of allele or genotype distribution between our samples and the general population in Taiwan. This suggests the schizophrenic patients with a specific genotype of ADRA1A gene might have a higher BMI (or obesity severity) than those with other genotypes of ADRA1A continuously after an exposure to antipsychotics therapy previously.

The recently identified type 2A juvenile haemochromatosis gene (*HJV*), a second candidate modifier of the C282Y homozygous phenotype. *G. Le Gac*^{1,2}, *V. Scotet*², *C. Ka*², *I. Gourlaouen*¹, *L. Bryckaert*¹, *C. Ferec*^{1,2}. 1) Etablissement Francais du Sang, Brest, France; 2) INSERM U 613, Brest, France.

The most common form of hereditary haemochromatosis is an adult-onset condition usually associated with the *HFE* C282Y/C282Y genotype. The phenotypic expression of this genotype is heterogeneous and depends on a complex interplay of genetic and non-genetic factors. The aim of the present study was to determine if mutations in the recently identified *HJV* gene were associated with more severe iron overload phenotypes in C282Y homozygous patients. From a cohort of 492 C282Y homozygous patients, DHPLC/sequencing of the whole *HJV* coding region detected 13 patients (9 males and 4 females) with an additional *HJV* missense mutation in the heterozygous state (S105L (n=2), E302K (n=3), N372D (n=2), R335Q (n=2) or the previously described L101P (n=3) and G320V (n=1)). At the time of diagnosis, iron indices were available for 9 patients and appeared to be more severe than those observed in C282Y homozygous patients of identical sex and similar age ranges. The mean serum ferritin concentration of the 6 males with an *HJV* mutation was significantly higher than that of C282Y homozygous males without an additional mutation (2350.3 [=1429.9] vs. 1227.2 [=1130.1] g/L; p=0.0233, Student *t* test). We have recently reported that mutations in the gene that encodes hepcidin (*HAMP*) could explain one part of the C282Y/C282Y-related phenotypic heterogeneity by accentuating the iron burden. Our new data reveals that mutations in the *HJV* gene could be associated with a similar effect. Taken together, these results emphasize that a search for modifier genes could enable us to more precisely distinguish those C282Y homozygous patients with a higher risk to develop a severe iron overload and, consequently, clinical complications.

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Association of SNPs within the serotonin transporter gene with depressive disorder and associated functional parameters. *S. Lucae, E.B. Binder, M.A. Ising, M. Uhr, B. Puetz, D. Salyakina, B. Mueller-Myhsok, S. Horstmann, P.G. Unschuld, M. Asmus, M. Koedel, F. Holsboer.* Max Planck Institute of Psychiatry, Munich, Germany.

The serotonin transporter gene promotor polymorphism (5-HTTLPR) has been described to be associated with response to treatment with SSRIs in patients with affective disorders. In this study we analyzed 8 SNPs throughout the SLC6A4 gene in 370 patients suffering from depressive disorder. We investigated gender differences, case/control associations, associations with response to antidepressant treatment, associations with neuropsychological items, as well as with neuroendocrinological data. We found no case/control association in the overall sample. However, when male patients with recurrent unipolar disorder were analyzed separately, we found a significance in 4 SNPs ($p=0.01$). Furthermore, we found an association regarding divided attention at admission and discharge in five SNPs ($p=0.001$) independent of gender differences. We found no association with response independent of the class of antidepressant treatment. 96 patients were treated with SSRI, 93 with mirtazapine and 64 patients were treated with tricyclic antidepressants. We could not find any significant effects regarding neuroendocrinological data emerging from combined dexamethasone-CRH tests. We are currently on the way to investigate SNPs in the promotor region and are going to determine 5-HTTLPR genotypes.

The brain-expressed tryptophan hydroxylase gene (TPH2) as a candidate gene for autism. *P. Malenfant*^{1,3}, *L.K. Waintraub*^{1,3}, *N. Novosedlik*^{1,3}, *X. Liu*^{1,3}, *J.J.A. Holden*^{1,2,3}, *ASD-CARC*⁴. 1) Department of Physiology, Queens University, Kingston, Ontario, Canada; 2) Department of Psychiatry, Queens University, Kingston, Ontario, Canada; 3) Autism Research Program, Ongwanada Resource Centre, Kingston, Ontario, Canada; 4) www.autismresearch.ca.

Autism is a severe neurodevelopmental disorder characterized by communication and social impairments, as well as repetitive interests and behaviours. Alteration of serotonergic pathways has been proposed to be part of the aetiology of a subgroup of cases of autism spectrum disorders (ASDs), making TPH, a gene encoding for the rate-limiting enzyme in the synthesis of serotonin, a good candidate gene for autism. With the recent identification of a second TPH isoform, TPH2, which is expressed in the brain, initial studies that attempted to link neurological disorders to TPH1 have to be revisited. Five TPH2 SNPs were genotyped in 288 affected siblings from 139 multiplex families, as well as in a comparison group of 190 randomly chosen newborns. The latter anonymous samples were obtained from the Ministry of Health of Ontario as heel prick blood spots for PKU screening. We performed case-control and family-based association studies, and examined the relationship between genotypes and phenotypes based on findings from the ADI-R. We found an increased G allele frequency for marker rs1487279 in families with at least one affected female (male-female and female-female families; $P=0.030$). In these families, the GG genotype was associated with an earlier age of first phrase ($P=0.025$). No significant results were obtained for the remaining 4 SNPs (-473A:T, rs1487279, rs1386492, rs1386494). The results suggest that there may be an association between autism and the TPH2 gene in the families with at least one affected female, but additional markers and families need to be examined to confirm this association. Supported by studentships from Queen's University, The Scottish Rite Charitable Foundation of Canada and Autism Society Ontario to PM and research grants from CIHR and OMHF to JJAH.

Multilocus analysis for the identification of at risk genotype in cardiovascular disease. *G. Malerba¹, E. Trabetti¹, M. Sandri², L. Xumerle¹, U. Cavallari¹, R. Galavotti¹, M. Biscuola¹, C. Patuzzo¹, P.F. Pignatti¹.* 1) Mother-Child & Biol, Genetics, Univ Verona, Verona, Italy; 2) CIDE - University of Verona, Verona, Italy.

The new methods are able to rapidly genotype many individuals for a large list of gene polymorphisms. Thus the complexity of the data is ever growing and the researches need to implement statistical methods that can reduce the complexity and target the relevant information. We developed an exploratory procedure implementing a classification tree-based recursive partitioning that aims to discover multigene patterns associated to the variability of the phenotype. The suggested marker-marker combinations were tested by logistic regression. The procedure was applied to a previously reported dataset of 1039 individuals characterized for coronary artery disease. Each individual was genotyped for 63 polymorphisms of 35 candidate genes. The noteworthy marker-marker patterns are: APOC3 -641 AA / APOC3 1100 CC (OR-corrected: 3.92, CI: 1.54-9.95, p=0.0041), APOC3 -455 TT / APOC3 1100 CC (OR-corrected: 3.83, CI: 1.50-9.76] p=0.00488), APOC3 -641 AA / ANP2238 TC/TT (OR-corrected : 3.53, CI: 1.15-10.79, p=0.0271), APOC3 -455 TT / ANP 2238 TC/TT (OR-corrected : 3.43, CI: 1.13-10.66, p=0.0295), ELAM128 ser-ser, ser-arg / LIPH 480 TT(OR-corrected: 0.14, CI: 0.03-0.66, p= 0.013). The polymorphisms APOC3 -641C/A and APOC3 -455T/C are tightly linked and were discordant in 34 individuals. Therefore they might be viewed as a single genetic factor carrying a similar information. The results indicate that multiplex genotyping can detect susceptibility polymorphisms and may help in finding possible gene-gene interactions associated to disease. Further studies should be performed to investigate the robustness of the method. Our results emphasize the need to account for complex multilocus influences when multifactorial diseases are studied. Acknowledgement: we thank S. Cheng, B. Rhee and HA. Erlich (Roche Molecular Systems, Inc., Alameda, CA, USA) for stimulating suggestions and for providing genotyping strips and reagents.

***parkin* mutations in a kindred with 4-generations of early-onset neuro-psychiatric disorders.** J.S. Montimurro¹, L. Moses¹, S. Factor², G.D. Schellenberg³, J.G. Nutt⁴, H. Payami¹. 1) Wadsworth Ctr., NYS Dept. of Health, Albany, NY; 2) Albany Medical Center, Albany, NY; 3) University of Washington VA Puget Sound Health Care System, Seattle, WA; 4) Oregon Health & Science University, Portland, OR.

parkin mutations, the most common known cause of Parkinsons disease (PD), are presumed recessive. We report transmission of *parkin* mutations over 4 generations. The proband with PD has a duplication (dup) of exons 2 & 3 (E2-3) and triplication (trip) of E4. To determine phase and configuration of the mutations we analyzed 11 relatives. Dosage was determined using semi-quantitative PCR on an ABI Prism 7000 Sequence Detection System using standard protocol. Samples were run in triplicate with -globin as the endogenous reference. Each assay was repeated four times, results averaged, and dosage assigned using standard cut-offs. We found 5 different mutant genotypes in 9 members of the same family: dup E2-3, trip E4 (proband, brother); del E2-3 (sister, niece); trip E2-E4 (sister, father, nephew); dup E2-4 (paternal grandmother); trip E2, 10 copies of E3, 17 copies of E4 (niece). Paternity and Mendelian transmission were established by haplotype analysis with 5 markers within and surrounding *parkin*. Using microsatellite haplotypes as a guide, the best explanation is 2 mutant chromosomes: dup E2-4 originating from the father and del E2-3 inferred to originate from the mother. The individual with multiple copies of E3 and E4 suggests de novo expansion. Of 12 individuals analyzed, 2 have compound mutations diagnosed with PD; 7 are heterozygous, 4 with parkinsonism, 3 teens unaffected; and 3 with no mutation are unaffected. Seven family members have psychiatric disorders: dementia, anxiety, depression, panic disorder, schizoid personality, alcoholism, drug abuse; 6 have mutation(s), 2 with PD, 4 with parkinsonism. This study demonstrates challenges in *parkin*. genotyping, the danger in misinterpreting test results without family data, and the lack of clear genotype-phenotype correlation. Novel findings include a possible link between *parkin*. and psychiatric disorders, and the possibility of de novo expansion/deletion in *parkin*.

Genetically determined dopamine levels are associated with obesity. *A.C. Need¹, K.R. Ahmadi¹, T.D. Spector², D.B. Goldstein¹.* 1) Department of Biology, University College London, London,UK; 2) Twin Research & Genetic Epidemiology Unit, St Thomas Hospital,London,UK.

The Western world is currently experiencing a pandemic of obesity, with a concomitant increase in associated factors such as heart disease, diabetes, osteoarthritis, and forms of cancer. Although this is clearly driven by lifestyle factors, family and twin studies have shown that BMI has a strong genetic component.

Human and animal studies have shown that DA is involved in appetite regulation, and here we have implicated a role for inherited reduction in dopamine (DA) metabolism in risk of obesity. We investigated functional polymorphisms in four genes that are responsible for DA reuptake and metabolism: COMT, DAT-1, MAOA and MAOB, in order to investigate how heritable variation in DA levels influences the risk of obesity. The COMT and DAT1 polymorphisms showed no association with either weight or BMI as quantitative traits, or with obesity in a case/control design. We found, however, that both MAOA and MAOB showed an excess of the low-activity genotypes in obese individuals (MAOA: $z = 15.45$, $p = 0.004$; MAOB: $z = 8.05$, $p = 0.018$). Additionally, the MAOA genotype was significantly associated with both weight ($p = 0.0005$) and BMI ($p = 0.001$) in a multiple regression model. When considered together, the at risk genotype - low activity genotypes at both the MAOA and MAOB loci - shows a relative risk for obesity of 5.01.

These results establish a connection between MAO activity and obesity. The fact that both MAOA and MAOB were associated suggests that this is mediated through their effects on DA metabolism. However they do not specify how DA levels influence obesity. We are therefore now assessing the relationship between MAOA and B and eating behaviours in a cohort of 8000 individuals, in order to confirm that these polymorphisms indeed result in heritable changes in eating behaviour.

TNF- G-308A polymorphism in patients with systemic lupus erythematosus from the western region of Mexico.

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Systemic lupus erythematosus (SLE) is considered multifactorial in its etiology. It can be regarded as a process that involves one or more environmental factors which act in a genetically susceptible individual. Over-expression of TNF- might be a significant risk factor for the development of SLE. The SNP G-308A in the promoter region of TNF- gene is related with the production of TNF- protein. It is known that allele A (TNF2) involves an increased transcriptional activity. Furthermore, TNF-, has been shown to be associated with autoimmune disorders, including SLE. The main objective of this study was to investigate the association between the G-308A polymorphism and SLE. This SNP was typed by PCR-RFLP in 73 individuals with SLE from the north-western region of the country, diagnosed according to the ACR criteria as well as in a control (C) group. This group consisted of 174 healthy volunteers living in Guadalajara, Mexico. In group C, allele TNF2 frequency was 10.1%, and genotype distribution was in agreement with HWE ($p>0.05$). In the group SLE, frequency of allele TNF2 was 17.1% ($p=0.03$ against group C), and genotype proportions were not different ($p>0.05$) from those of group C. This study's results are consistent with the idea that allele TNF2 is associated with the development of SLE in north-western Mexicans via increased TNF- levels.

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Mutation analysis of RELN as a candidate gene for schizophrenia. *J. Saldivar, J.S. Muci, J. Feng, P.H. Burkhart, J. Yan, S.S. Sommer.* Dept Clinical Molecular Gen, City of Hope, Duarte, CA.

Schizophrenia is a common psychiatric disorder that displays non-Mendelian genetic inheritance. The glycoprotein reelin is secreted by GABA-ergic neurons during neural development, and several lines of evidence implicate reelin in the pathogenesis of schizophrenia. To test the hypothesis that mutations in the large reelin gene (RELN) may predispose to schizophrenia, coding exons and associated splice junctions of the RELN gene were analyzed in 64 patients with schizophrenia and 80 controls using the DOVAM-S followed by direct sequencing. DOVAM-S is a robotically enhanced, multiplexed, and highly redundant form of SSCP that detects virtually all mutations within a scanned genomic segment. A total of 5.1 Mb of the RELN genomic sequence was scanned. Multiple missense mutations occurring at highly conserved amino acids were identified, as well as different silent and intronic sequence changes. A dramatically high frequency of variants was found in African-American individuals. There was a non-significant excess of structural variants in schizophrenia relative to controls, warranting study in a larger sample.

Phenotype-genotype studies: the clue for unraveling the genetics of psychiatric disorders? *T.G. Schulze^{1, 2, 3}, S. Ohlraun^{1, 2}, J. Schumacher⁴, L. Kassem³, T. Becker⁵, J. Hauser⁶, S. Wellek¹, S. Cichon⁷, F.A. Henn¹, F.J. McMahon³, W. Maier², P. Propping⁴, M.M. Nöthen⁷, M. Rietschel^{1,2}.* 1) CIMH, Mannheim; 2) Dept of Psychiatry, Univ Bonn; 3) MAPGenetics, NIMH, Bethesda, MD; 4) Inst Hum Genet, Univ of Bonn; 5) IMBIE, Univ Bonn; 6) Dept of Adult Psychiatry, Univ Poznan; 7) Dept Med Genet, Univ Antwerp.

We have previously reported association between the G72/G30 locus and both schizophrenia and bipolar disorder (BD) in large samples of German cases and controls. A specific 4-marker risk haplotype confers risk to both disorders. Given the presumed role of G72/G30 in the neurochemistry of psychosis and its localization in a region (13q34) linked to schizophrenia and BD, we hypothesized the BD finding to be mainly due to an association with psychotic features. We performed a case-control analysis in the subset of 173 patients with delusions and/or hallucinations. Odds ratios increased only marginally. We were therefore interested in whether confining case definition to specific psychotic content might be better at detecting a potential genotype-phenotype correlation between BD and G72/G30. Using a logistic regression analysis on 21 OPCRIT items for delusional or hallucinatory content, we found the item lifetime history of persecutory delusions to be the only significant ($p=0.0047$) explanatory variable for the G72/G30 risk genotype. We then tested for association between G72/G30 and BD in the subset of patients with a history of persecutory delusions ($n=90$). We observed significant changes in the odds ratios (maximum increase from 1.33 to 1.83; $p=0.0024$) for the G72/G30 markers/haplotypes, compared to the overall sample of 300 BD patients. The same risk haplotype was associated in an independent BD sample from Poland when case definition was restricted to cases with persecutory delusions, while there was no association in the overall sample. Our data suggest that BD with persecutory delusions constitutes a distinct subgroup of BD and support the idea of a genetic overlap between schizophrenia and BD. Further analysis is needed to elucidate whether persecutory delusions per se is important or rather some other trait that correlates with it.

Progression of atherosclerosis in coronary artery bypass grafts is associated with the HMG-CoA Reductase gene.

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3-hydroxy-3-methylglutaryl-Coenzyme A reductase (HMGCR) is the rate-limiting enzyme for cholesterol synthesis and the target of statin drug therapy to reduce plasma LDL-cholesterol. The national Post-Coronary Artery Bypass Graft (post-CABG) Trial demonstrated that an aggressive regimen to lower LDL-cholesterol using statin therapy reduced, but did not eliminate, the progression of atherosclerosis in grafts. The aim of this study was to test whether HMGCR polymorphisms were also associated with the progression of atherosclerosis in grafts (progression). DNA was collected and atherosclerosis in grafts was measured using angiography at baseline and 4-5 years later in 843 subjects from the Post-CABG trial. These subjects were also genotyped for 5 HMGCR polymorphisms (mean spacing 4.1kb) using TaqMan MGB technology. Associations were tested with logistic regression (SAS). Analysis with HaploView demonstrated that all 5 SNPs formed one haplotype block. Haplotype 4 (H4, frequency 5.8%) was associated with a higher percentage of subjects showing the progression of atherosclerosis in grafts (progression in 46% of H4/- subjects vs 36% all other genotypes, $p=0.017$) while H2 (frequency 29%) was associated with a lower percentage of subjects showing progression (progression in 25% of H2/H2 subjects vs 39% all other genotypes, $p=0.0023$). There was a significant effect of H4/- or H2/H2 on the number of grafts with progression per subject ($p=0.0038$ and $p=0.0048$, respectively, adjusted for age, sex, BMI). No interaction between HMGCR variation and aggressive or moderate statin therapy was observed, nor did this effect appear to be mediated by LDL-cholesterol level. These results suggest that variation in HMGCR may influence the progression of atherosclerosis post-CABG by pathways in addition to those involving the LDL-cholesterol lowering effect of statins.

Criteria for evaluating the validity and utility of endophenotypes in psychiatric genetics: Application to executive functions and childhood ADHD. *I.D. Waldman.* Psychology, Emory University, Atlanta, GA.

There is renewed interest in psychiatric genetics in endophenotypes, constructs posited to be more directly and strongly influenced by relevant genes than manifest disorder. Several researchers have proposed criteria for the selection of endophenotypes that might be useful in finding genes that underlie psychiatric disorders and their symptoms. These criteria include: 1. good psychometric properties, 2. relation to the disorder and its symptoms in the population, 3. expression regardless of whether the disorder is currently present, 4. expression at a higher rate in the unaffected relatives of probands than in randomly selected individuals from the population, 5. the endophenotype and disorder are associated within families (i.e., they co-segregate), 6. the endophenotype is heritable, 7. common genetic influences underlie the endophenotype and the disorder, 8. the endophenotype must show association and/or linkage with one (or more) of the candidate genes or genetic loci that underlie the disorder, 9. the effects of a gene on a disorder are expressed either in full or in part through the endophenotype (i.e., the endophenotype *mediates* the gene-disorder relation), and 10. the effects of a particular gene or locus on a disorder are stronger in disordered individuals who also show the endophenotype (i.e., the endophenotype *moderates* the gene-disorder relation). In this talk, I review some of the analyses that may be used to evaluate the validity and utility of putative endophenotype measures consistent with these proposed criteria. I illustrate the use of such analyses to evaluate putative endophenotypes with data on childhood ADHD and executive function measures from both a candidate gene study of clinically-referred children with ADHD and a study of non-referred twins. I demonstrate that while the putative endophenotype measures showed association with several candidate genes in the dopamine system, and met many of the criteria proposed for their validity and utility, they did not meet all of the criteria and might have been rejected for use as endophenotypes in molecular genetic studies on that basis.

Genetic Analysis of DYX1C1 in native English speaking cohort. *K. Womble¹, E. Dawson¹, D. Sawyer².* 1) Innovative Projects, BioVentures, Inc., Murfreesboro, TN; 2) Center for Dyslexic Studies Middle Tennessee State University, Murfreesboro, TN.

Developmental dyslexia is considered a complex trait affecting up to 15% of the population. In order to find candidate genes that may be involved in developmental dyslexia, numerous linkage studies have shown linkage among several chromosomes including 15q21, specifically the DYX1C1 gene. We studied the DYX1C1 gene by sequencing all exons and flanking intronic regions in a native English speaking cohort that was tested to be either dyslexic or non-dyslexic from the Middle Tennessee area and were unable to reproduce the association found in the Taipale et al. (2003) DYX1C1 study. We found 8 single nucleotide polymorphisms, four of which coincide with the previous findings and three which were novel. One novel SNP found was located at chromosome location 15:53447790 causing a non-synonymous amino acid change Ile 276 Val that lies within a tetratricopeptide repeat domain (TPR) of DYX1C1. No significant associations of the polymorphisms which we observed in DYX1C1 were found in the dyslexic cohort studied when considered individually or as haplotypes. Therefore, our results do not support evidence for variations of the candidate gene DYX1C1, on chromosome 15q21 to be associated with dyslexia in our cohort. We conclude that population genetic and language culture differences contribute to the complexity of discovering and validating candidate genes for dyslexia.

Analysis of the 2q33 region in celiac disease. *K. Brophy¹, A.W. Ryan¹, C. Feighery¹, C. O'Morain¹, N. Kennedy², R. McLoughlin¹, F. Stevens³, D. Kelleher¹, R. McManus¹.* 1) DMMC and Trinity College, Ireland; 2) Dept. Medicine, Trinity College, Ireland; 3) NUI, Galway, Ireland.

Chromosome region 2q33 contains the immune regulatory genes, Cytotoxic T-Lymphocyte Antigen 4 (CTLA4), Inducible Co-Stimulator (ICOS) and CD28, making this region an interesting candidate for investigation in autoimmune disease. CTLA4 has previously been implicated in several autoimmune diseases including celiac disease (CD). Our aim was to establish whether an association exists between this region and CD in the Irish population using a comprehensive analysis for genetic variation. 393 celiac disease patients and 345 ethnically matched healthy controls were typed for the five htSNPs in CTLA4, seven SNPs in ICOS and three SNPs in CD28. Haplotype-tagging SNPs were predominantly chosen. Haplotype frequencies were estimated using computational analysis and allele, genotype, carrier status and haplotype frequencies were compared between populations. The CTLA4658 SNP was the only polymorphism found to show significant association, after correction for multiple testing using the Bonferroni method, between populations when allele, genotype, or carrier status frequency were analysed (carrier status (Allele C), $p=0.0017$). CTLA4+49, which has previously been implicated in CD in other populations, was not significantly associated in this study ($p=0.1072$). The CT60 SNP was included in the variants tagged by the haplotype-tagging approach of the CTLA4 gene. Using this method, we found CT60 to be marginally associated with disease ($p=0.0190$) prior to correction. Linkage disequilibrium analysis identified a block of LD incorporating the CD28 and CTLA4 polymorphisms and this CD28/CTLA4 haplotype was significantly associated with CD (patients 24.4%; controls 31.1%; $p=0.0061$). Our results suggest that CTLA4658 or a genetic variant in LD with CTLA4658 may be associated with disease. Furthermore, the haplotype block which includes the CD28/CTLA4 genes shows significant association with disease which is stronger than when either gene is analysed individually. This suggests that a causal variant associated with this haplotype may be linked with CD in this population.

Genetic Variation Upstream of NPY2R is Associated with Obesity in Men. *C. Campbell*^{1,2}, *H. Lyon*¹, *S. Purcell*³, *M. Daly*³, *L. Groop*⁴, *D. Altshuler*^{2,3,5}, *K. Ardlie*⁶, *J. Hirschhorn*^{1,2,3}. 1) Divisions of Genetics and Endocrinology, Childrens Hospital, Boston, MA; 2) Department of Genetics, Harvard Medical School; 3) MIT Broad Institute; 4) Department of Endocrinology, University Hospital MAS, Lund University, Malmö, Sweden; 5) Department of Molecular Biology, Massachusetts General Hospital; 6) Genomics Collaborative, Inc., Cambridge, MA.

Obesity is a growing worldwide epidemic. Approximately 50% of the variation in body mass index (BMI), a measure of obesity, is due to genetic factors, but the genetic variants that influence obesity remain unknown. We have studied the genes encoding three related peptides (NPY, PYY, and PPY) as well as their receptors (NPY1R, NPY2R, and NPY5R) for association with obesity. These peptides regulate appetite and food intake, and some of the corresponding genes are in regions of linkage to obesity. We defined the patterns of common variation in these genes in a sample of 96 chromosomes from 12 multigenerational pedigrees of European origin (CEPH). We then selected haplotype-tagging SNPs (htSNPs) that capture most of the common variation, and genotyped these htSNPs in patient populations. We used two large case-control studies, a US Caucasian panel and a Polish panel, consisting of cases (1200 US, 700 Poland) from the 90th-97th percentile in BMI and controls (600 US, 330 Poland) from the 5th-12th percentile in BMI. No statistically significant results were found for NPY, PYY, PPY, NPY1R, or NPY5R in the combined panels. Interestingly, five SNPs 0.6-5.0 kb upstream of the NPY2R gene showed a nominally significant association with obesity in men in both samples (combined $p=0.001$, odds ratio=1.34, 95% confidence interval, 1.12-1.59) for the best SNP). These results were replicated (one-tailed $p=0.034$, odds ratio=1.20) in men from 240 Scandinavian parent-offspring trios. The combined p -value for all three European-derived panels is 3.1×10^{-4} (odds ratio=1.27 95% CI, 1.12-1.45). The SNPs were not significantly associated with obesity in women. These results suggest that common variation in the NPY2R gene influences the development of obesity in men; we are in the process of attempting to further replicate this result in additional samples.

Haplotype analysis of SNPs in the CNR1 gene and association with progression in Marijuana Use. M.A.

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Background: A number of adoption, twin, and family studies have indicated that genetic factors contribute to marijuana use and dependence. However, the specific genes contributing to a persons risk of becoming dependent on cannabis remain unknown. The cannabis receptor (CNR1) in addition to being expressed in the brain, is the principal site of action of delta-9-tetrahydrocannabinol (THC), the principal psychoactive ingredient in marijuana. This project involved utilizing the genomics resources to examine SNPs in the human CNR1 gene. **Methods:** 558 subjects participating in existing studies of substance abuse were selected for a pilot association study, on the basis that they had tried marijuana prior to age 16 and were at least age 17 or older at the last assessment (thus, allowing at least one year between initiation and progression). Approximately half of these individuals exhibited at least one DSM-IV dependence symptom for cannabis. TaqMan Assays-on-Demand from Applied Biosystems were used to genotype four SNPs within the CNR1 gene in all subjects. Using GOLD, the D measure of linkage disequilibrium between pairs of SNPs was determined to vary between 0.10 and 0.99 (G. Abecasis; <http://www.sph.umich.edu/csg/abecasis/GOLD>). We also used WHAP (S. Purcell; <http://www.broad.mit.edu/personal/shaun/whap>), a SNP haplotype analysis package, to test individual SNPs and haplotypes for possible associations with the phenotype of at least one dependence symptom. **Results:** CNR1 haplotypes, but not individual SNPs, were associated with progression in cannabis use. **Conclusions:** Further work in this area should contribute to the understanding of the underlying genetic and biological mechanisms contributing to progression of marijuana use. This work has been supported by the NIH/NIDA P60 DA 11015-05, DA015522, and DA000357.

Allele-specific transcript quantification detected haplotypic variation in the levels of the SDF-1 transcripts. R.

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The *SDF1*-G801A, a SNP in the 3' untranslated region of the *SDF1* gene, has been reported to be associated with susceptibility to diseases such as AIDS and type-I diabetes by epidemiological studies probably through the altered levels of SDF-1 production. However, experimental studies of the effect of the G801A have not supported its functional importance. To examine whether other polymorphisms have *cis*-acting effects on *SDF1* expression, we carried out a haplotype analysis of the *SDF1* gene and the allele-specific transcript quantification (ASTQ) using Epstein-Barr virus-transformed lymphoblastoid cell lines. After a screening of SNPs in the *SDF1* gene, we selected 10 haplotype-tagging SNPs and genotyped for them. The haplotype phase were estimated from the genotype data by the PHASE program. For the ASTQ, we utilized the G801A polymorphism as a tag to distinguish each of the allele-specific transcripts and examined their proportion in each cell lines with heterozygous genotype for this polymorphism. A haplotype-based analysis of the ASTQ revealed that G+6201A in the intron 2 and C-668G in the promoter region are independently associated with the altered levels of the transcripts. In addition, another polymorphism in the promoter region, C-1652T, was associated with the different levels of response to the dibutyl cyclic AMP treatment that enhances *SDF1* transcription. These results suggested that other polymorphisms than the G801A do have functional roles in SDF-1 production. Providing evidence for the haplotypic variation in the levels of the transcripts, this study contributes to improving interpretation of the previous disease-association studies and selection of SNP markers for future studies. We show that the ASQT based on haplotype information is a powerful means to detect the *cis*-acting effect of polymorphisms on gene expression.

Association of DISC1 and bipolar disorder. *K. MAEDA*^{1,3}, *J. Chang*¹, *R. Youn*¹, *R. Kawahara*³, *M. McInnis*¹, *A. Sawa*^{1,2}. 1) Psychiatry, Johns Hopkins University, Baltimore, MD; 2) Neuroscience, Johns Hopkins University, Baltimore, MD; 3) Psychiatry, Tottori University, Japan.

Disrupted in Schizophrenia-1 (DISC1) was identified at the breakpoint of a balanced translocation segregating with schizophrenia and mood disorder in a large Scottish family. Additional findings of association between DISC1 and schizophrenia have been reported, but not with mood disorder. We conducted a family-based association study of DISC1 and bipolar disorder in 57 bipolar pedigrees. We performed direct sequences with 10 samples from the patients with bipolar disorder (BP) and identified a novel (unreported) SNP. Twelve SNPs were genotyped at the DISC1 locus, including the novel SNP, using the Taqman assay. The computer program FBAT was used for family-based TDT and haplotype analysis. Association at individual SNP was observed at rs3737597 in 3'-UTR of DISC1 gene ($P < 0.05$). Haplotype analysis identified one haplotype (HP1) that was overtransmitted to the BP phenotype ($P = 0.01$) and a second haplotype that was undertransmitted (HP2). There was evidence of gender influence in the transmission distortion, with overtransmission of HP1 to affected females ($P = 0.004$). Expression levels of DISC1 in lymphoblasts were compared between affected subjects with HP1 and unaffected subjects with HP2. There was a significant decrease in DISC1 expression in affected HP1 group compared to cell lines derived from unaffected subjects with the HP2 ($P = 0.006$). This difference was more pronounced in females ($P = 0.001$). These results suggest an association between DISC1 and bipolar disorder, and a decreased level of expression of DISC1 in lymphoblastoid cell lines from affected female subjects with the risk haplotype.

DNA repair polymorphisms and survival from bladder cancer in men. *G. Matullo*^{1,2}, *S. Guarrera*¹, *S. Polidoro*¹, *S. Gamberini*^{1,2}, *C. Sacerdote*³, *F. Saletta*¹, *L. Davico*³, *L. Fiorini*³, *D. Fontana*⁴, *A. Piazza*², *P. Vineis*^{1,3,5}. 1) ISI Foundation, Torino, Italy; 2) Dept Genetics, Univ Torino, Italy; 3) CPO Piemonte, Univ Torino, Italy; 4) Cattedra di Urologia, Univ Torino, Italy; 5) Imperial College London, UK.

In industrialized countries bladder cancer is the fifth most common cancer in men. The removal or repair of DNA damage have a key role in protecting the genome from the insults of cancer-causing agents. Polymorphisms in DNA repair genes have been identified and extensively studied in relation to the modulation of cancer risk, but few studies have been conducted on the relationship between genetic polymorphisms and survival from cancer. In the context of a survival study on 364 incident cases of transitional-cell bladder cancer, we investigated the survival status in relation to 13 polymorphisms in seven DNA repair genes (XRCC1-28152A/G, XRCC1-26651A/G, XRCC1-26304T/C, XPD-23591G/A, XPD-35931A/C, XRCC2-31479G/A, XRCC3-18067C/T, XRCC3-17893A/G, XRCC3-4541C/T, ERCC4-30147A/G, ERCC4-30028C/T, PCNA-6084G/C, ERCC1-19007C/T). All cases are men aged 40-74 followed up for mortality for an average of 65 months (range 0.36-126.24, median 48m, SD 37.6): of them, 90 died and 274 were surviving at the end of follow-up. According to univariate analysis, none of the analyzed polymorphisms showed statistically significant results considering a proportional hazard model though, after adjustment for age, stage, and grade, ERCC1-CC genotype was significantly associated with a worse prognosis (CC vs TT, HR=3.8, 95% CI=1.1-13.3). Histological grade and clinical stage are significantly associated with survival both at univariate (grade $p=0.00011$; stage $p=0.000046$) and age corrected multivariate analysis (grade G1=ref; HR_{G2}=8.2, 95% CI 1.0-63.4; HR_{G3 or >G3}=13.8 95% CI 1.5-123.5; stage A=ref., HR_{PT1}=1.3, 95% CI 0.6-3.0; HR_{PT2}=1.0, 95% CI 0.2-3.2; HR_{>PT2}=6.2, 95% CI 1.7-23.2). No evidence of a worse prognosis according to XRCC3, XRCC1, XPD/ERCC1 and ERCC4 genes estimated haplotypes has been obtained, except for the rare XRCC1-TAA haplotype ($p=0.015$). Our results support a limited impact of DNA repair polymorphism on bladder cancer survival.

DRD3 gene polymorphisms: significant observations in Parkinson's disease in India. *S. L.Punia*¹, *G. Nainwal*², *V.M. Khare*², *M. Behari*³, *U.B. Muthane*⁴, *B.K. Thelma*⁵, *R.C. Juyal*². 1) University of Delhi South Campus, New Delhi, India; 2) National Institute of Immunology, New Delhi, India; 3) Department of Neurology, All India Institute of Medical Sciences, New Delhi, India; 4) Department of Neurology, NIMHANS, Bangalore, India.; 5) Department of Zoology, University of Delhi, Delhi, India.

The dopaminergic pathway genes have been implicated for the etiology of Idiopathic Parkinsons disease (IPD) and also as drug induced adverse effects. DRD3, a D2 like receptor is an important gene in dopaminergic pathway and one of major targets of L-dopa and dopamine agonists. We have investigated the possible role of multiple polymorphisms in DRD3 gene in the pathogenesis of PD using a case-control approach. Three SNPs, (one in the promoter, one exonic, and one intronic) in DRD3 gene were genotyped by PCR-RFLP method in a total of 515 IPD subjects diagnosed according to UK PD brain bank clinical diagnostic criteria and 374 age, sex, and ethnicity matched population based controls. There is no allelic and genotypic association of any of these three markers with PD. Haplotypes were constructed separately for cases and controls using program PHASE2.0.2 and tested for global haplotypic significance using program CLUMP. We observed highly significant ($\chi^2=154.45$, $df=7$, $p\lll 0.05$) global haplotypic association with PD, which remain significant even after Bonferroni Correction for multiple comparisons. Susceptibility conferred by individual haplotype(s) was assessed by computing p value and Odds Ratio (OR) at 95% confidence interval (95% CI) of one haplotype vs. the rest. We observed highly significant deviation in the distribution of some of the haplotypes between cases and controls. Thus DRD3 gene polymorphisms may be a major genetic determinant in the etiology of PD in Indian population. Our results also reiterate the importance of analyzing SNP haplotypes vs. individual SNPs in association studies.

Simulations to evaluate sample stratification results an example using *TNFA* and schizophrenia. V. Saviouk¹, E.W.C. Chow², A.S. Bassett², L.M. Brzustowicz^{1,3}. 1) Dept Genetics, Rutgers Univ, Piscataway, NJ; 2) Dept of Psychiatry, Univ of Toronto, and Clinical Genetics Research Program, Centre for Addiction and Mental Health, Toronto; 3) Dept of Psychiatry, Univ of Medicine and Dentistry of NJ, NJ Medical School, Newark.

Previously we showed the association of *TNFA* H1 (-308A, -238G) haplotype with schizophrenia (SZ) by the Trimhap test ($p=0.0052$). Upon stratification of the data set based on the *TNFA* H1 haplotype there were several loci in the genome that showed linkage to SZ that were not detected in the initial scan (Brzustowicz *et al* Science, 2000). We have now applied simulation methods to assess the significance of these results.

Simulate was used to generate 1,000 replicates of full autosomal scans. Parametric linkage analysis was performed on each of the replicates. The pedigrees were analyzed as a single group, then divided into two subsets, based on the presence ($n=16$) or absence ($n=6$) of the *TNFA* H1 haplotype in the real data. In each subset, the maximum LOD and HLOD scores were recorded for each marker and, if ≥ 3.0 , were compared to the maximum LOD/HLOD values at the same marker in the whole replicate. For each replicate, the maximum increase in LOD/HLOD observed for all scores ≥ 3.0 was recorded for each of the two subsets. These values were organized to empirically evaluate the increase in LOD/HLOD scores in the real data.

Several loci showed interesting increases in LOD scores. However, only a single new locus, D1S1609 on 1q44, demonstrated a significant increase ($p=0.025$) in LOD score from 0.15 to 3.01, analyzed with a broad SZ phenotype and a dominant mode of inheritance. This replicates a positive result of linkage of SZ spectrum disorders to this area (Ekelund *et al* Hum Mol Genet, 2001). H1 *TNFA* haplotype partitions our sample into two parts, one with linkage to 1q44 (H1-) and one with the previously reported linkage to 1q22 (H1+) that did not exhibit any evident changes in significance upon stratification. We showed that simulations are pivotal in evaluating the results when sample stratification is utilized.

Screening of genes related with arachidonic acid metabolism for the susceptibility of aspirin induced asthma. T.

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In a subset of asthmatic patients, aspirin and several other non-steroidal anti-inflammatory drugs (NSAID) that inhibit cyclooxygenase (COX) enzymes induced severe asthmatic attack generally termed aspirin induced asthma (AIA). We investigated allelic association of 370 single nucleotide polymorphisms (SNPs) of 63 candidate genes, mostly from arachidonic acid metabolic cascade, to AIA. After two rounds of screening with 198 AIA patients, multiple SNPs in prostaglandin E2 receptor subtype 2 (EP2) gene were associated with AIA ($P < 0.05$). 77 SNPs were identified in EP2 gene, and we applied 17 SNPs based on linkage disequilibrium and allelic frequencies (minor allele frequency > 0.1) for further association study. SNPs in the promoter region of EP2 gene, uS5, uS5b, and uS7, were significantly associated with AIA ($P = 0.039-0.001$). Haplotype analysis was undertaken and a significant association with AIA was observed ($P = 0.001$). The most significantly associated SNP, uS5, locating in the regulatory region of EP2 was in a STATs-binding consensus sequence. However, the STAT1 binding was not observed in gel mobility shift assay with HeLa nuclear extract, instead, an unidentified protein was specifically bound to the variant sequence. *In vitro* reporter assay in HCT116 cells, the site containing the uS5 variant showed a reduced transcription activity. Taken together, the uS5 variant serves as target for a repressor protein of transcription. The functional variant of EP2 gene associated with the risk of AIA would decrease the transcription of EP2 resulting in the reduction of the PGE2 braking mechanism of inflammation and thus could be implicated in the molecular mechanism underlying AIA.

Haplotype Analysis Of Four HTR1B Polymorphisms In Obsessive-Compulsive Disorder. *T.L. Sicard¹, P.D. Arnold¹, J. Geronimo¹, G.L. Hanna², M. Pato³, M.A. Richter¹, J.L. Kennedy¹.* 1) Neurogenetics, Centre for Addiction and Mental Health, Toronto, Ontario, Canada; 2) University of Michigan, Ann Arbor, Michigan, USA; 3) VA Medical Center, Washington, DC, USA.

Obsessive-Compulsive Disorder (OCD) occurs in 1-3% of the US population and is among the ten leading causes of disability worldwide. It is characterized by obsessions, which are recurring and persistent undesirable thoughts or images, and compulsions, which are necessities to perform excessive rituals or repetitive acts in order to reduce anxiety. Genetic factors are believed to play a major role in the etiology of OCD, and disturbances of the serotonergic pathways have been implicated in this disorder. In the past, researchers have focused mainly on a single nucleotide polymorphism or SNP (861G/C) in the 5-HT-1D-beta receptor (HTR1B) gene, the results have been inconsistent. The aim of the present study was to examine genetic association between OCD and multiple polymorphisms in HTR1B in 85 nuclear families from Toronto, Ann Arbor and Washington. Four SNPs in HTR1B were examined: the common 861G/C SNP, a promoter SNP (-161A/T) shown to affect transcriptional activity and two additional SNPs located at the 5' (rs1213371) and 3' (rs200292) untranslated regions of the gene. Results were obtained for analysis of the haplotypes using Transmit program, however, no statistically significant haplotype associations were identified. Our results suggests that the HTR1B gene does not confer susceptibility for OCD in our sample, however, genotyping of additional SNPs for HTR1B in a larger sample of OCD families may be required in order to exclude the involvement of this gene.

Allelic and Expression Analyses of the Human Lipin 1 Gene in Finnish Dyslipidemic Families. *E. Suviolahti¹, K. Reue², RM. Cantor², A. Soro-Paavonen³, MR. Taskinen³, P. Pajukanta², L. Peltonen¹.* 1) Dept Molecular Medicine, National Public Health Institute, Helsinki, Finland; 2) Dept Human Genetics, David Geffen School of Medicine at UCLA, University of California, Los Angeles, CA; 3) Department of Medicine, University of Helsinki, Finland.

Dysfunction of the lipin 1 (*Lpin1*) gene is the cause of lipodystrophy, hypertriglyceridemia and insulin resistance in the fld mouse. The role of lipin 1 (*LPIN1*) in humans is so far unknown. We investigated seven intragenic single nucleotide polymorphisms (SNPs) and four microsatellite markers of the human *LPIN1* gene in 1109 individuals (538 males, 571 females) from 92 Finnish dyslipidemic families ascertained for a proband with either low high-density lipoprotein cholesterol (HDL-C) or familial combined hyperlipidemia (FCHL). The SNPs and their haplotypes were analysed for association with the critical traits that are abnormal in the fld mouse such as fasting serum triglyceride (TG), glucose and insulin levels as well as body mass index (BMI) and postheparin plasma activities of lipoprotein lipase (LPL) and hepatic lipase (HL), using the family-based association test (FBAT). In addition, *LPIN1* mRNA expression levels were determined in fat tissue of 19 individuals from low HDL-C and FCHL families and analysed for correlation with BMI and serum TG, glucose and insulin levels. Our analyses of *LPIN1* gene variants in dyslipidemic families revealed suggestive evidence for association with serum glucose and insulin levels as well as with LPL activity ($P < 0.05$). Furthermore, in females the most significant association was detected for HL activity ($P < 0.005$). *LPIN1* gene expression levels in fat tissue correlated with plasma levels of serum glucose ($r^2 = 0.40$) and insulin ($r^2 = 0.32$). In line with the results obtained previously from the mouse *Lpin1* gene, these results suggest that the human counterpart, *LPIN1*, may also have a role in the regulation of the glucose and insulin metabolism. This study also emphasizes the importance of murine models in the understanding of genetically complex human traits.

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Untranslated Region Haplotype in Dysbindin Gene. Analysis in Schizophrenia. *D.Z.A. Voineskos, V. De Luca, T. Shinkai, G. Wong, J. Trakalo, J.L. Kennedy.* Neurogenetics, Centre for Addiction and Mental Health, Toronto, Ontario, Canada.

Several studies have supported the existence of many susceptibility genes of small effect for schizophrenia. Genome-wide linkage scans performed in schizophrenia families have provided evidence for region 6p24-21 where variability may confer susceptibility to schizophrenia. Recent studies have implicated that the DTNBP1 gene (dysbindin) in this region is strongly associated with schizophrenia. In a family association study of 116 families, we investigated three markers located in the 3 untranslated regions of the DTNBP1 gene, by genotyping with Assay by Design (ABI Applied Biosystems, Foster City, USA): the BfaI polymorphism (SNP 909706: an AG transition), rs1047631 (an AG transition), and rs742106 (a CT transition). We hypothesize that these three markers are functionally related and are more likely to affect the DTNBP1 expression than other intronic haplotypes that have previously been studied. Haplotype analysis was done using TRANSMIT, but the global chi-squared value was not significant ($\chi^2 = 4.8074$, $df = 3$, $p = 0.186477$). The hypothesis for DTNBP1 in schizophrenia remains of high interest, and more markers need to be typed at this gene to provide more detailed haplotype information and thus a more detailed test of this candidate locus.

Association between LMX1B haplotypes and primary open-angle glaucoma in individuals of African descent. A.

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Glaucoma is a blinding neurodegenerative disease that disproportionately affects individuals of African descent. Mutations in the LMX1B transcription factor are responsible for Nail-Patella Syndrome, which includes glaucoma as a variable feature. Here, we investigate the role of polymorphisms in LMX1B in susceptibility to non-syndromic primary open angle glaucoma (POAG) in subjects of African and European descent. We sequenced the 1119 bp coding region, 3' UTR, and flanking intronic regions in 155 subjects with POAG and 101 matched controls. 147 subjects are of African descent, 99 are of European descent. We found 23 polymorphisms in the LMX1B gene, 16 with a minor allele frequency >1%. We also found differences in allele and haplotype frequencies between the two populations, and therefore analyzed them separately. Initial single SNP association tests identified four associated SNPs ($p < 0.05$) in samples of African descent, all four SNPs map to intron 3. To compare haplotype frequencies between cases and controls, we estimated haplotype frequencies using the expectation-maximization algorithm. We analyzed each population separately and estimated significance levels by permuting case and control labels for all subjects. In individuals of African descent, we find significant differences in haplotype frequencies between individuals with POAG and unaffected controls. For example, considering the 16 common SNPs, one haplotype with a frequency of 9.42% occurred only in cases but not in controls ($p = 0.009$). In addition, there are significant differences in the distribution of rare haplotypes (frequency <5%) between the case and control group. In summary, our data provide evidence for association between haplotypes in LMX1B and POAG in individuals of African ancestry.

Allelic variants of the -aminobutyric acid B receptor subunit 2 (GABABR2) gene are associated with nicotine dependence. *J. Beuten*¹, *J.Z. Ma*¹, *T.J. Payne*³, *R.T. Dupont*², *K.M. Crews*³, *M.D. Li*¹. 1) Dept Psychiatry, UTHSCSA, San Antonio, TX; 2) The University of Memphis; 3) The University of Mississippi Medical Center.

Tobacco use is a major worldwide health problem, being responsible for 20% of all premature deaths; nearly one-third of the global adult population smokes. Nicotine dependence (ND) is a complex trait with strong genetic and environmental influences, although the susceptibility genes remain largely unknown. We genotyped 12 single nucleotide polymorphisms (SNPs) in the GABAB receptor subunit 2 gene (GABABR2) for association with ND in a sample of 600 families comprising an extensively phenotyped population of more than 1,400 smokers and non-smokers of both African- and Caucasian-American origin. The GABABR2 gene is a strong candidate for involvement in ND because GABA, the main inhibitory neurotransmitter in the brain, is involved in many neurochemical pathways that regulate physiological and psychological processes. Furthermore, the gene is located within a region on chromosome 9q22 that showed significant linkage to ND in our previous genome-wide screening. Single SNP analysis using PBAT-GEE indicated that 4 of the 12 SNPs covering the GABABR2 gene were significantly associated with smoking behavior in the African-American population, and 3 of the 12 SNPs showed significant results in the Caucasian-American population. Of interest is that the exonic SNP rs3750344 showed significant association with smoking-related phenotypes ($p = 0.000015$ in the Caucasian-American samples; $p = 0.0178$ in the African-American samples). Haplotype analysis using FBAT provided evidence for a positive association of the rs2491397(G)-rs2184026(A)-rs3750344(C) haplotype with smoking-related phenotypes in the overall sample ($Z = 4.02$; $p = 0.000058$). In summary, this study not only confirms previous data on the importance of the family of GABA receptor genes in addiction behavioral traits but provides insight into the genetics of ND, showing the importance of future studies of the GABABR2 gene as a critical candidate in regulating smoking behavior (Supported by DA-12844).

A variant within an intron of RAPTOR (regulatory protein of mTOR) at 17q25 is a modifier of PSORS1 (HLA class I) in psoriasis. *A.M. Bowcock, C. Helms, P. Taillon-Miller, N.L. Saccone, B. Pierce.* Genetics Dept., Wash. Univ. School Med., St. Louis, MO.

Psoriasis is a chronic inflammatory skin disease affecting ~2% of the Northern European population. Its frequency is elevated in HIV patients and 20-30% of patients have psoriatic arthritis. A major determinant of psoriasis maps to the HLA class I region (PSORS1), although its identification has been elusive. Moreover, its penetrance is only ~15% indicating a requirement for genetic modifiers or environmental triggers. PSORS2 maps to chromosome 17q25, and two peaks of association where TDT $p < 0.001$ lie within this region, separated by 6 Mb. The first peak harbors SLC9A3R1 and NAT9; genes involved in polarized epithelia/the immune synapse and acetylation respectively. One predisposing allele within this region and lying between the two genes leads to loss of RUNX1/AML1 binding (Helms et al. 2003). The second peak lies within an intron of RAPTOR (regulatory protein of mTOR). We have replicated the association with RAPTOR in an independent set of families from the U.K. This association is strongest when cases with a family history of disease are used (Capon et al. 2004). Recent analyses have revealed epistasis between RAPTOR and PSORS1: PDT p value for a previously associated marker within RAPTOR increased in significance from 0.009 to 0.0007 when trios were selected on the basis of cases harboring a variant upstream from HLA-C. These findings indicate that the RAPTOR-associated variant is a modifier of PSORS1, and also illustrates the power of epistasis studies to identify causative variants in regions with complex LD patterns such as the MHC.

Association study of CREB1 and childhood-onset mood disorders. *I. Burcescu¹, K. Wigg¹, N. King², A. Vetro³, E. Kiss³, L. Katay³, J.L. Kennedy², M. Kovacs⁴, C.L. Barr^{1,5}, The International Consortium for Childhood-Onset Mood Disorders.* 1) Cell and Molecular Division, The Toronto Western Hospital, Toronto, ON, Canada; 2) Neurogenetics Section, Centre for Addiction and Mental Health, Toronto, Ontario, Canada; 3) Department for Child and Adolescent Psychiatry, Szeged University, Szeged, Hungary; 4) University of Pittsburgh, Pittsburgh, Pennsylvania, USA; 5) The Hospital for Sick Children, Toronto, Ontario, Canada.

Several lines of evidence suggest that the cellular pathways involved in synaptic plasticity contribute to the risk of depression including the finding that chronic antidepressant treatment up-regulates the cAMP signal transduction cascade resulting in increased expression and function of the cAMP responsive element binding protein (CREB), a transcription factor that increases the expression of key growth factors involved in synaptogenesis and neurogenesis. Recently, linkage was reported for early-onset depression (onset < 25 years) to 2q33-35, in the region of the gene for CREB1, in families recruited from the Pittsburgh area. This finding was significant only in female sibling pairs from those families. Further, two specific DNA variants, -656G/A and a cytosine insertion/deletion in intron 8, were identified in this gene that co-segregated with depression in two of the families. We sought to replicate this finding by testing for an association between CREB1 and childhood-onset mood disorders (COMD) in a sample of 195 nuclear families (225 affected children) collected in Hungary. We genotyped the two CREB1 DNA variants previously identified as linked to depression as well as three additional polymorphisms spanning the gene. In addition, we genotyped the -656G/A DNA change and insertion/deletion polymorphism in intron 8, in a sample of 112 cases and matched controls collected in the Pittsburgh area, and examined the distribution of alleles and genotypes. The results of our study show no evidence for an association between COMD and CREB1, and no gender-specific transmission for any of the polymorphisms tested.

Association of TNF-, Corneodesmosin, SEEK1 genes with Psoriatic Arthritis. *C. Butt¹, F. Siannis², V. Farewell³, F. Pellett², L. Peddle⁴, C. Schentag², D. Gladman², B. Fernandez¹, P. Rahman¹.* 1) Faculty of Medicine, Memorial University of Nfld., St. John's, Newfoundland and Labrador, Canada; 2) University of Toronto; 3) Institute of Public Health, Cambridge UK; 4) Newfoundland Genomics, St. John's, Newfoundland and Labrador, Canada.

The MHC region has long been considered a strong candidate region for an underlying PsA susceptibility gene. In view of the location and proposed biologic effect of TNF-, corneodesmosin (CDSN), and SEEK1, we set out to determine if polymorphisms within these genes are associated with PsA in a founder and admixed population from the same country. We assessed PsA patients and ethnically matched healthy controls from a founder and an admixed population for the TNF, CDSN and SEEK1 SNPs. PsA subjects and controls were genotyped for TNF-, SEEK1, and CDSN by time-of-flight mass spectrometry. All five TNF- SNPs were in the 5 flanking region of TNF- gene at the following positions: -1031 (TC), -863 (CA), -857 (CT), -308 (GA) and -238 (GA). The following four SNPs were tested in the CDSN gene 619 (rs707913); 1215; 1236 (rs1042127); 1243 (rs3132554), and SNP +39604 of SEEK1. All primers were designed using Sequenom SpectroDESIGNER software, scanned using a mass spectrometry workstation (Bruker), and analyzed using the Sequenom SpectroTYPER-RT software. Results: There was no association observed between the CDSN SNPs and onset of psoriasis, PsA or arthritis pattern. An association between SEEK1 and PsA was noted in the founder population (OR 2.0, p=0.017); however by using logistic regression we determined that SEEK1 does not appear to be a further susceptibility factor if the HLA-Cw*0602 status is already known, and is likely in linkage disequilibrium with HLA-Cw*0602. A modest association was noted between the TNF- 238 A allele and PsA in the founder population (OR 2.5, p=0.02) which was not validated from the admixed cohort. None of the other SNPs tested were noted to have an association between TNF/CDSN/SEEK1 and onset of psoriasis, PsA or arthritis pattern in either population.

Association study of the spinocerebellar ataxia 1 gene located at 6p22.3 that show linkage with schizophrenia and bipolar disorder in multigenerational families from Eastern Quebec. *Y.C. Chagnon, C. Merette, M. Cayer, J. Lamarche, M-A. Roy, M. Maziade.* Molecular Psychiatry, Robert-Giffard Research Center, Beauport, Quebec, Canada.

BACKGROUND. Significant linkages with schizophrenia (SZ) at 6p24-p22 have been reported repeatedly in different populations. A risk haplotypes of the dysbindin (DTNBP1) gene located at 6p22.3 have been associated with SZ. We have observed a linkage (Lod=3.47) with SZ at 6p22.3 one centimorgan centromeric to DTNBP1. Our linkage directly involved the marker SCA1 located within the spinocerebellar ataxia 1 (ATX1) gene associated to the olivopontocerebellar atrophy. In a previous study, ATX1 has been shown to be also in linkage disequilibrium with SZ. **OBJECTIVE.** To evaluate a possible association with SZ and BP of the ATX1 microsatellite used in our linkage study. **SAMPLES.** 45 multigenerational families (~1 000 family members) with multiple cases of SZ and bipolar disorder (BP). 300 unrelated SZ and 150 controls. **METHODS.** Non-parametric linkage analysis, factorial analysis, and chi square and variance analysis tests. **RESULTS.** We observed potential differences between SZ and controls in genotype distribution ($p=0.0002$) with a borderline result for allele distribution ($p=0.07$), as well as between BP and controls ($p=0.03$ and $p=0.11$, respectively). Genotype and allele distributions in SZ appeared to be different of BP ($p=0.01$ and $p=0.04$, respectively). Four single nucleotide polymorphisms (SNP) located within DTNBP1 showed no significant differences ($p>0.09$) between the SZ, BP and control subjects, or between the 300 unrelated SZ patients and 150 controls ($p>0.30$). Using our factor analysis to derive continuous variables from symptoms of SZ and BP, three factors representing negative symptoms, psychosis, or depression, respectively, were detected. We compared the mean scores on these factors by ATX1 genotype using a variance analysis. A significant difference was observed between SZ and BP patients ($p=0.003$) underlying the ATX1 genotype effect on certain clusters of symptoms. This supports the difference in genotype and allele distribution observed for SZ in contrast to BP in our families.

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Gene expression analysis in bipolar disorder using lymphoblastoid cell lines. *H. Chen, C.A. Ross, R. DePaulo, Jr., M.G. McInnis.* Psychiatry & Behavioral Sci, Johns Hopkins Univ Sch Med, Baltimore, MD.

Microarray analysis has made genome-wide testing gene expression feasible but the need for appropriate brain tissue has placed a serious limit on transcriptome-wide testing in complex disorders of the brain. To explore a potential strategy to surmount this limit, we examined the expression profiles in lymphoblastoid cell lines from bipolar I subjects (BP I) and corresponding unaffected first-degree relative controls on the Affymetrix Genechip platform (U133 plus2.0). Data analysis was performed using the Spotfire DecisionSite and Functional Genomics software. The mean expression value of each gene was determined in 11 BP I subjects and 11 unaffected controls. We analyzed the genes detected present in all 22 cell lines, and found that there were 70 genes with 1.3 fold changes or greater associated with a t-test p-value < 0.05 in BP I subjects compared to controls. Ten genes were selected for real-time quantitative RT-PCR verification using the TaqMan method. Nine genes showed similar changes (> 1.3 fold) in the BP I subjects that is consistent with microarray findings, however, only 3 were statistically significant using a two-tailed t-test. Our study suggests that lymphoblastoid cell lines are a potential powerful and accessible cellular system for the analysis of genes and expression in psychiatric disorders where availability of brain tissue is limited.

***MECP2* variation in Portuguese autistic patients.** A.M. Coutinho¹, G. Oliveira², C. Katz³, J. Feng³, J. Yan³, C. Yang³, C. Marques², A. Ataíde², T.S. Miguel², T. Temudo⁴, P. Maciel⁵, S.S. Sommer³, A.M. Vicente¹. 1) Instituto Gulbenkian de Ciência, Portugal; 2) Hospital Pediátrico de Coimbra, Portugal; 3) City of Hope National Medical Center and Beckman Research Institute, USA; 4) Hospital de Sto. António, Portugal; 5) Escola de Ciências da Saúde, Universidade do Minho, Portugal.

Mutations in the gene encoding the methyl-CpG-binding protein 2 (*MECP2*) are responsible for Rett syndrome (RTT). Autism is a neurodevelopmental disorder that shares symptoms with RTT, and therefore an association of *MECP2* with autism etiology as been questioned. Some *MECP2* mutations described in RTT have also been reported in autistic patients. In this study, the *MECP2* gene was scanned for mutations in a Portuguese autistic sample, to assess if the phenotypic spectrum of mutations can extend beyond the traditional diagnosis of RTT and X-linked mental retardation syndromes, possibly leading to a milder phenotype in males. The coding region and 3'untranslated region (3'UTR) were scanned in 172 autistic children and 143 controls, by detection of virtually all mutations-SSCP (DOVAM-S). Although the role of the 3'UTR is not clearly defined, this region is unusually long in *MECP2* and well conserved between human and mouse, suggesting an important regulatory role. We report a novel G206A missense change, found in an autistic boy with severe mental retardation and absence of language. The missense change leads to an alteration in aminoacid properties, which is likely to alter the secondary structure of the protein. Moreover, it's located in a highly conserved nucleotide inside a region implicated in an alternative transcriptional repression pathway, and therefore it is highly possible that this change leads to a functional alteration of MeCP2. Thirteen new 3'UTR variations not present in the controls, eight of which located in conserved nucleotides, were also found. The present results support the hypothesis that *MECP2* underlies several neurodevelopmental disorders, and that distinct mutations alter differently the MeCP2 function, leading to a diversity of clinical manifestations due to the differential abnormal expression of its target genes.

Assessing STRK1 and STRK2 in our independent cohort. *E. Evans*¹, *J. Meschia*², *M. Brown*³, *S. Rich*³, *S. Hague*¹, *R. Brown*², *B. Kissela*², *T. Brott*², *J. Hardy*¹, *A. Singleton*¹. 1) Lab of Neurogenetics, National Institute on Aging, Bethesda, MD; 2) Dept. of Neurology, Mayo Clinic, Jacksonville, FL; 3) Department of Public Health Sciences and Neurology, Wake Forest University School of Medicine, Winston Salem, NC.

A phosphodiesterase 4D (PDE4D) haplotype on chromosome 5q12 was associated with an increased risk of ischemic stroke last year. Earlier this year the same group published an additional risk locus for stroke, within the gene ALOX5AP, which encodes the 5-lipoxygenase activating protein (FLAP) on chromosome 13. We tested these two loci for linkage and association to stroke using two study designs, a concordant sib pair series and a case/control association series. The series comprised individuals age 18 years or older who either presented with ischemic stroke within 180 days after the onset of symptoms or were found to be neurologically normal. The sample series consisted of the SWISS series (Siblings with Ischemic Stroke Study), the ISGS series (Ischemic Stroke Genetic Study) and the PRE-SWISS series, totaling over 600 samples. To test their findings, we used the markers and SNPs previously linked to disease. For PDE4D, we typed markers AC008818-1, SNP-32, -45, -56, -83 and -87 and for ALOX5AP, we ran microsatellite markers D13S289 and D13S1238 and SNPs SG13S89, SG13S32, SG13S25, and SG13S114. For both loci we analyzed microsatellite markers using an ABI3100, and for each SNP we either designed a restriction digest assay or used an ABI Assay-by-Design allelic discrimination assay. After performing statistical analyses on each of the sample series for each marker, our data suggests that no significant linkage or association was identified in the SWISS, ISGS or PRE-SWISS series with any of the markers tested. Although both PDE4D and ALOX5AP were found to be associated with ischemic stroke in an Icelandic cohort, in a more diverse sample series, the two genes do not appear to associate strongly with disease.

The Adrenergic System and Attention-Deficit/Hyperactivity Disorder. *Y. Feng¹, K. Wigg¹, J. Crosbie², T. Pathare², A. Ickowicz², R. Tannock², W. Roberts², M. Malone², J.L. Kennedy³, R. Schachar², C.L. Barr^{1,2}.* 1) Cell & Molecular Biology Division, The Toronto Western Hospital, University Health Network; 2) The Hospital for Sick Children; 3) Neurogenetics Section, Centre for Addiction and Mental Health.

Considerable evidence suggests the involvement of noradrenergic mechanisms in attention-deficit/hyperactivity disorder (ADHD). The noradrenergic (norepinephrine) system is known to be involved in visual attention, modulation of vigilance (sustained attention), initiation of adaptive response, learning and working memory. Elegant work using both animal models and pharmacological probes has shown that the adrenergic system and particularly the alpha 2 adrenergic receptor, are critical for maintenance of visual-spatial information. Therefore the noradrenergic system may contribute more specifically to the cognitive deficits observed in ADHD, particularly working memory. Using a sample of 186 nuclear families including 50 siblings, we investigated the genes for the adrenergic receptors ADRA1A, ADRA1B, ADRA1C, ADRA1D, ADRA2A, ADRA2B, and ADRB1, ADRB2, ADRB3 as candidates for susceptibility to ADHD. Multiple markers across each gene were examined. First, we analyzed ADHD as a categorical trait using transmission disequilibrium test (TDT) analysis; we then applied a quantitative approach family based association test (FBAT) to examine symptom dimensions of inattention, hyperactivity/impulsivity and digit span forwards and backwards-as a proxy for working memory. Preliminary results indicate lack of evidence for a relationship between these genes and ADHD diagnoses, nor did we find any significant relationship between inattentive and hyperactive/impulsive traits and these genes. However, we did observe some preliminary evidence for an association with markers in the ADRA2A gene and working memory.

A Class II Cytokine Receptor Gene Cluster is a Major Locus for Hepatitis B Persistence. *A.J. Frodsham¹, L. Zhang¹, U. Dumpis², N. Mohd-Taib³, S. Best¹, A. Durham³, H. Whittle², H.C. Thomas³, M.R. Thursz³, A.V.S. Hill¹.* 1) The Wellcome Trust Centre for Human Genetics, Oxford, UK; 2) Medical Research Council, Fajara, The Gambia; 3) Imperial College School of Medicine, London, UK.

There are 350 million people with persistent hepatitis B virus (HBV) infection worldwide. Of these up to one third will die from complications of the infection such as cirrhosis and primary liver cancer. Furthermore current treatment for persistent HBV virus infection is poorly effective, expensive and impractical in developing countries where rates of persistence are at their highest. Host genetics have been shown to play a role in the outcome of infection. To further elucidate the role of host genetic factors in the resolution of infection, we performed a genome wide scan in a large set of Gambian families with children with persistent HBV infection. The most significant region of the genome found to exert a major effect on susceptibility to persistence was found on chromosome 21. This area contains a cluster of important genes belonging to the cytokine receptor 2 family. Polymorphisms resulting in amino acid changes in two of the genes within this cluster, the 2nd subunit of the interferon alpha receptor (IFNAR2) and the interleukin 10 receptor beta subunit (IL10RB), are significantly associated with the outcome of HBV infection in this population. Furthermore, haplotype analysis also shows a highly significant correlation with disease persistence. Additionally, we have evidence that these non-conservative amino acid substitutions have a direct functional effect on cell surface expression levels and work is now underway to clarify the exact nature of the role of this variation with respect to HBV persistence. Both of these genes are known to play a central role in host immunity and thus provide us with new avenues for identifying novel therapies for HBV infection for use in endemic areas where it will be most beneficial.

Polymorphisms in trace amine receptor 4 (TRAR4) are associated with susceptibility for schizophrenia on chromosome 6q23.2. *P.V. Gejman¹, J. Duan¹, A.R. Sanders¹, C. Hou¹, N. Saitou², T. Kitano², J. Comeron³, B. Mowry⁴, D.F. Levinson⁵, R.R. Crowe⁶, J.M. Silverman⁷, M. Martinez⁸.* 1) Dep. Psychiatry, ENH RI/ Northwestern University, Evanston; 2) Div. Population Genetics, National Institute of Genetics, Mishima, Japan; 3) Dept. Biological Sciences, The University of Iowa, Iowa City; 4) Dept. Psychiatry, University of Queensland, Brisbane, Queensland, Australia; 5) Dept. Psychiatry, The University of Pennsylvania, Philadelphia; 6) Dept. of Psychiatry, University of Iowa College of Medicine, Iowa City; 7) Dept. of Psychiatry, Mount Sinai School of Medicine, NY; 8) Méthodologie Statistique et Epidémiologie Génétique des Maladies Multifactorielles, Institut National de la Recherche et de la Santé Médicale, Evry, France.

Several linkage studies provide convergent support for chromosome 6q13-q26 as containing a locus for schizophrenia, and more recently for bipolar disorder. We genotyped 192 schizophrenia pedigrees across the MOXD1-STX7-TRARs gene cluster at 6q23.2, which contains a number of prime candidate genes for schizophrenia. Thirty-one screening SNPs were selected, providing a minimum coverage of at least 1 SNP per 20 kb. The association observed with rs4305745 ($P = 0.0014$) within the TRAR4 (trace amine receptor 4) gene remained significant after correction for multiple testing. Through database searching and sequencing genomic DNA in a 30 probands sub-sample, we obtained a high-density map of twenty-three SNPs spanning 21.6 kb of this gene. Single SNP and also haplotypic analyses revealed that rs4305745, and/or 2 other SNPs in perfect linkage disequilibrium (LD) with rs4305745, appears the most likely mutation underlying the association of the TRAR4 region with schizophrenia. Comparative genomic analyses revealed that rs4305745, and/or the associated SNPs in complete LD, can affect gene expression. Moreover, RT-PCR studies of various human tissues including brain confirm that TRAR4 is preferentially expressed in those brain regions implicated in the pathophysiology of schizophrenia. These data provide strong preliminary evidence that TRAR4 is a susceptibility gene for schizophrenia and await replication attempts in additional clinical samples.

Screening for mutations in exon 1 of the Rett syndrome gene, MECP2, among individuals with autism and with mental retardation. *C. Harvey*¹, *S.E. Alfred*¹, *G.N. Mnatzakanian*², *S.W. Scherer*², *W. Roberts*³, *B.A. Minassian*², *J.B. Vincent*¹. 1) Neuroscience Research, CAMH, Toronto, Ontario, Canada; 2) Program in Genetics and Genomic Biology, Hospital for Sick Children, Toronto, Ontario, Canada; 3) Childhood Developmental Centre, Hospital for Sick Children, Toronto, Ontario, Canada.

We recently identified and reported a new isoform of the Rett syndrome gene, MECP2, which results in a new coding region within the first exon. We also reported the identification of a mutation in a single female Rett patient within this new coding sequence- an 11 base pair deletion that results in a frameshift, causing a premature truncation of the protein, and thus supporting a role for this new isoform in the etiology of Rett syndrome. Previously, mutations in the MECP2 gene (exons 3 and 4) have also been identified in individuals with other neuropsychiatric disorders, including autism, mental retardation and Angelman syndrome. We hypothesized that, since this new coding region is clearly important for the correct function of MECP2 during neurodevelopment, this coding region would also be relevant for screening in cases of autism and mental retardation (and non-15q11 cases of Angelman). We have now screened this region in ~100 autism probands, and in ~100 patients with mental retardation. Direct DNA sequencing was used for mutation detection. No mutations were identified among the autism screening set, however 4 sequence variants were identified among the MR cases. We report here on the sequence variants identified, and their possible relevance to neurodevelopment and mental retardation.

Expression analysis in Parkinson Disease (PD), progressive supranuclear palsy (PSP), and frontotemporal dementia with Parkinsonism. *M. Hauser¹, M. Nouredine¹, R.W. Walters¹, C.M. Hulette¹, D.E. Schmechel¹, M.L. Bembe¹, J.M. Stajich¹, H. Xu¹, E.R. Martin¹, B.L. Scott¹, J. Stenger¹, Y-J. Li¹, R. Jensen³, C. Scherzer², S. Gullans², J.M. Vance¹.* 1) Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Brigham and Womens Hospital, Harvard Medical School, Boston, MA; 3) Department of Physics, Wesleyan University.

Parkinsons Disease (PD) is a progressive neurodegenerative disease characterized by the loss of dopaminergic (DA) neurons in the substantia nigra. We present gene expression profiling in the substantia nigra of six patients with PD, two with progressive supranuclear palsy (PSP), one with frontotemporal dementia with Parkinsonism (FTDP), and five controls. PSP and FTDP are related disorders that, like PD, show loss of DA neurons this allows us to identify expression changes that may be secondary to cell loss. All samples were analyzed with Affymetrix U133A microarrays, and a subset was analyzed by serial analysis of gene expression (SAGE). Genes from several metabolic pathways are significantly ($p < 0.05$) altered in PD (but not PSP and FTDP) patients. The molecular chaperones HSPA1A and HSPA1B are overexpressed. There are significant reductions in vesicle transport genes: 9 subunits of the vacuolar ATPase ATP6 are reduced, as are GDI1, STXBP1, SYT1, and VAMP1, proteins involved in docking of secretory vesicles. Mitochondrial gene expression is substantially altered: of the top 20 SAGE tags most significantly differentially expressed between PD and control, five map to the mtDNA. Overall, our SAGE data reveal a 1.5-fold increase in mtDNA-generated tags in PD SN compared to control. This increase was not matched by an increase in the number of tags that map to nuclear genes encoding mitochondrial proteins, indicating a possible disruption of mitochondrial homeostasis. Differentially expressed genes that map to regions of PD linkage are being investigated as susceptibility genes: family-based and case/control association analysis of tightly-linked polymorphisms will be presented.

Identification of Candidate Genes for Clubfoot. *J.T. Hecht*^{1,2}, *A. Heck*³, *M. Bray*³, *S. Watlington*¹, *A. Scott*², *S.H. Blanton*⁴. 1) Univ of Texas Medical School, Houston; 2) Shriners Hospital for Children, Houston, TX; 3) Univ of Texas School of Public Health, Houston; 4) University of Virginia, Charlottesville.

Idiopathic talipes equinovarus (ITEV), or more commonly, clubfoot, is a developmental deformity that is characterized by a rigid foot, adducted forefoot, cavus midfoot, equinovarus of the hindfoot, and hypoplastic calf musculature. This common birth defect is treatable but the etiology of ITEV is largely unknown. We have collected a large sample of ITEV probands and their families, including simplex trios, trios with a positive family history, and extended multiplex families, for use in genetic studies. We have tested two groups of candidate genes for linkage with and without association in this population. The first group consisted of CASP8, CASP10, and CFLAR. These apoptosis regulators were chosen because they map to the chromosomal region 2q31-33 which has been identified as significantly associated with translocations in ITEV. Typing of SNPs throughout the genes revealed positive linkage with association with a variant in CASP10 in the simplex trios ($p < 0.003$). The second group of candidate genes consisted of NAT1 and NAT2. These genes were chosen because they are involved in tobacco metabolism and smoking has been identified as a risk factor for ITEV. Of the 5 polymorphisms tested, only one in NAT2 yielded significant results ($p = 0.03$) under an additive model in the simplex trios. The polymorphisms in NAT2 are known to act in a recessive manner, with only individuals who have a variant form for both copies of the alleles having an altered acetylation rate. Therefore, a recessive model was also considered. The NAT2 polymorphism remained significant, with a p-value of 0.007, with a deficit of the normal allele. These results suggest that CASP10 and NAT2 may play a role in the etiology of ITEV. We are examining CASP10 for functional sequence changes. In addition, the CASP10 results provide a scientific foundation to further explore the contributions of other apoptotic genes in the etiology of clubfoot. The NAT2 results are especially important results as they have significant implications for pregnant women who smoke during pregnancy.

Characterization of an Osteoporosis Linkage Peak: A Case/Control study to validate genes targeted by BoneFusion modeling software. *K.E. Irenze¹, K. Ardlie¹, B. Jordan¹, J. Lemaire¹, P. Enrietto², L.T. Herren².* 1) Genomics Collaborative, a division of SeraCare Life Sciences, Inc., Cambridge, MA; 2) Kenna Technologies, Inc., West Chester, PA.

Osteoporosis is a common disease that affects both men and women as they age. One of the best predictors of risk for osteoporosis is bone mineral density (BMD). A linkage peak for low bone mineral density in the hip was identified on chromosome 1p36 (Devoto et al. 1998, *Eur J Hum Genet*, 6 151-157). This linkage peak contained nearly 100 genes spanning over 5 Mb of sequence. Our goal was to determine whether in silico analysis of putative candidate genes within molecular pathways, prior to testing those candidates, can enable us to more accurately target higher-likelihood candidates and reduce both the cost and time involved in identifying the genes associated with disease. To this end, Kenna Technologies applied BoneFusion to enhance the evaluation and prioritization of a subset of gene candidates for targeted analysis. BoneFusion knowledge diagrams and simulations were used to provide evidence for a role for these genes in bone remodeling and to evaluate the effects of modulating activity/levels of candidate genes on BMD through BoneFusion simulation. Seven genes were selected for further analysis including resequencing and association studies on samples from the GCI Global Repository. Resequencing of these genes, in 48 Osteoporosis Cases, resulted in discovery of 44 novel SNPs not previously reported in public databases. A cohort of 728 USA white/caucasian, non-Hispanic Cases was selected, and all Cases have confirmed Osteoporosis with at least one DEXA Scan T-Score reading of 2.5 or less, in addition to full spine and full hip Z-Scores, T-Scores, and BMD measurements available. All Cases were individually matched with healthy Controls having no Osteoporosis for at least one generation. Analysis of 130 single nucleotide polymorphisms that were selected and genotyped on the 728 Cases and 728 Controls will be presented.

Mutation, selection and evolution of the Crohns disease susceptibility gene, CARD15. *K. King¹, M. Sheikh¹, A.P. Cuthbert¹, S.A. Fisher¹, C.M. Onnie¹, M.M. Mirza¹, J. Sanderson², A. Forbes³, J. Mansfield⁴, C.M. Lewis¹, R.G. Roberts¹, C.G. Mathew¹.* 1) Dept Genetics & Development, GKT School of Medicine, London, UK; 2) Dept Medicine, GKT School of Medicine, London, UK; 3) St Marks Hospital, Harrow, UK; 4) Dept Gastroenterology & Hepatology, University of Newcastle, UK.

Crohns disease (CD) - a chronic inflammation affecting the gastrointestinal tract - is associated with mutations in the CARD15 gene. There is also evidence of an excess of rare CARD15 variants in CD. Mutation analysis of the coding region of CARD15 identified many missense variants of 5% frequency. The evolutionary conservation of CARD15 was investigated to help assess the candidacy of rare variants as potential disease susceptibility alleles (DSAs). CARD15 was sequenced in three non-human primates and bioinformatic analysis was performed to identify other orthologues, and the conservation of amino acid substitutions examined. Seven rare variants (R393H, A612T, R703C, R830Q, N852S, R896X and R1019G) were classified as potential DSAs and these were genotyped in 600 cases and 600 controls. The allele frequency of R703C was 0.6% in cases and 0% in controls ($p=0.0019$). The low frequency of the remainder did not allow formal statistical testing. Phylogenetic analysis of CARD15 revealed ambiguity concerning the N-terminus of the translation product. Human, chimpanzee and gibbon CARD15 all appear to possess an in-frame ORF upstream of the putative start site in rat, mouse, zebrafish and pufferfish, potentially coding for a further 27 amino acids. Use of both translation initiation sites (81 nucleotides apart) has previously been identified in human CARD15. Interestingly, we observed a homozygous frameshifting insertion in tamarins that would preclude use of the first translation initiation site. This particular species of tamarin (*Saguinus oedipus*) is susceptible to a spontaneous chronic colitis which has served as an animal model for related human diseases. We are currently testing constructs for expression in 293T cells to assess the functional significance of the primate-specific upstream open reading frame of CARD15, and the potential consequences of its disruption in tamarins and related New World monkeys.

Association of the SNAP25 gene with bipolar affective disorder and suicidality. T.A. Klempan¹, A.H.C. Wong², T. Armstrong¹, V. Deluca^{1,2}, C.L. Barr^{2,3}, F.M. Macciardi^{1,2}, J.L. Kennedy^{1,2}. 1) Neurogenetics Section, Centre for Addiction and Mental Health, University of Toronto, Toronto, ON, Canada; 2) Department of Psychiatry, Faculty of Medicine, University of Toronto, Toronto, ON, Canada; 3) Toronto Western Hospital, Toronto, ON, Canada.

The SNAP25 gene encodes one of three functionally conserved presynaptic molecules (SNAREs) comprising a core complex controlling generalized exocytosis and neurotransmission. Altered levels of the protein have been identified in several psychiatric conditions, as well as in rodent models of these disorders. Importantly, based upon the phenotype of the coloboma mouse (with a deleted Snap25 gene) and association studies, a link has been made between variation at this locus and attention deficit hyperactivity disorder (ADHD). We have investigated association of three transitions in the 3 untranslated region of SNAP25 within 283 nuclear families with bipolar affective disorder in conjunction with diagnosis and attempts at suicide, a frequent consequence of affliction with this disorder. Analyses conducted using TDT demonstrate significant overtransmission of the *DdeI* 1 allele to female probands ($\chi^2=4.654$, $P=0.031$). The family-based association test (FBAT) also suggests biases in allele transmission under a recessive model (*DdeI* 1 allele: $Z=1.694$, $P=0.090$; *MnII* 1 allele: $Z=1.821$, $P=0.069$), while the most frequent SNAP25 haplotype shows differential transmission ($\chi^2=4.72$, 1df, $P=0.030$). An effect of two of these variants on the likelihood of attempted suicide was observed for a subset of the probands using ANCOVA ($F[2,102]=3.327$, $P=0.040$ for *DdeI* polymorphism, $F[2,102]=2.470$, $P=0.090$ for *MnII* polymorphism), further pronounced when stratified by allele presence or absence ($F[1,103]=6.533$, $P=0.012$ for *DdeI* polymorphism; $F[1,103]=4.196$, $P=0.040$ for *MnII* polymorphism). These results suggest an association of SNAP25 sequence variants with both bipolar disorder and suicidal behavior, possibly through disequilibrium with further mutations affecting level of expression or function in neurotransmission. Further investigation of molecules governing synaptic vesicle fusion and release may be warranted in studies of suicide.

Case-control association studies of 122 candidate genes for idiopathic Parkinson's disease. *I. Mizuta^{1,2}, W. Satake¹, Y. Momose³, Y. Hirota¹, A. Oka⁴, H. Inoko⁴, M. Yamamoto⁵, N. Hattori⁶, M. Murata⁷, T. Toda^{1,2}.* 1) Div Functional Genomics, Osaka Univ Grad Sch Med, Osaka, Japan; 2) CREST, JST, Saitama, Japan; 3) Dept CBI, Grad Sch Med, Univ Tokyo, Tokyo, Japan; 4) Dept Mol Life Sci, Tokai Univ Sch Med, Kanagawa, Japan; 5) Dept Neurol, Kagawa Pref Central Hosp, Kagawa, Japan; 6) Dept Neurol, Juntendo Univ Sch Med, Tokyo, Japan; 7) Dept Neurol, NCNP, Tokyo, Japan.

Idiopathic Parkinson's disease (PD) is a complex disorder caused by multiple genetic and environmental factors. To disclose the genetic factors, we are doing case-control analysis by using single nucleotide polymorphisms (SNPs) in multiple candidate genes. We selected candidate genes from the viewpoints of familial PD, dopaminergic neurons, Lewy body, trophic factors, cytokines, oxidative stress, mitochondria, apoptosis, ubiquitin-proteasome, autophagy, etc. For initial screening, we genotyped 190 patients and 190 controls by Invader method. Of 267 SNPs in 122 candidate genes, 22 SNPs in 16 genes showed P values less than 0.05 (allele frequency, chi-square statistics). These included UCHL1, NDUFV2, CTSD, FGF2, BCL2, etc. We are now confirming these associations by increasing the number of samples to nearly 900 for patients and 900 for controls.

AQP1, selected by differential screening of gene initiation sequences, is associated with schizophrenia in Portuguese population. X. Ni^{1,2}, J. Trakalo¹, J. Valente³, M. Azevedo³, M. Pato⁴, C. Pato⁴, J. Kennedy^{1,2}. 1) Neurogenetics Section, Ctr Addiction & Mental Health, Toronto, ON, Canada; 2) Department of Psychiatry, University of Toronto, Canada; 3) Department of Psychiatry, University of Coimbra, Portugal; 4) Veterans Administration Hospital, Washington, D.C., USA.

Using differential screening of gene initiation sequences (GIS), the DNA sequences that surround the gene start codon, we identified a product, showing stronger signal in a pool of probands with schizophrenia compared with their parents. The sequence of this DNA fragment shows high homology with the aquaporin 1 gene (AQP1), which is located on human chromosome 7p14 and codes the aquaporin 1 (channel-forming integral protein, 28kDa). To confirm the association between AQP1 and schizophrenia we tested four markers in the AQP1 gene with TaqMan technique in 163 Portuguese trios and nuclear families with DSM-IV schizophrenia probands. The program HaploView (version 2.04) from the Whitehead institute was used to test for concordance with Hardy-Weinberg equilibrium (HWE), calculate the linkage disequilibrium (LD), create the LD blocks, and perform the transmission disequilibrium test (TDT). TDT for extended marker haplotypes (TRANSMIT version 2.5.2) was used to test for transmission disequilibrium between haplotypes of the four AQP1 polymorphisms and schizophrenia. The genotype distributions in probands were all in HWE ($p > 0.05$). In TDT analysis using each allele we found a significant association between schizophrenia and the marker rs2267719 in AQP1 ($p = 0.0285$), and a trend to significance with the polymorphisms rs2267720 ($p = 0.0588$). The markers rs2267719 and rs2267720 of AQP1 were in high linkage disequilibrium. In haplotype transmission analysis the rs1859840A-rs2267719C-rs1049305G of AQP1 transmitted often than the expected ($p = 0.0305$) though the global chi-square test showed a negative result ($p = 0.1137$). Our results suggest that the human AQP1 may be associated with schizophrenia.

The MTHFR polymorphism is associated with acute rejection in kidney transplantation. *W.S. Oetting¹, M.J. Brott¹, N. Li², W. Pan², R. Kandaswamy³, A.J. Matas³.* 1) Dept. Medicine and Institute of Human Genetics, Univ. Minnesota, Minneapolis, MN; 2) Dept. of Biostatistics; 3) Dept. of Surgery.

Introduction: Several DNA polymorphisms have been previously associated with acute rejection (AR) after transplantation. We have now expanded this work with the analysis of 17 polymorphisms in 14 genes to determine their association with AR in our own population. **Methods:** DNA was isolated from 301 recipients, all of whom received Ab induction and calcineurin inhibitors (CNI) with either mycophenolate mofetil (MMF) or sirolimus. 17 single nucleotide polymorphisms (SNPs) were genotyped including ACE (I/D), AGT (p.T174M and p.M235T), F2 (c.20210G>A), F5 (p.R506Q), F7 (p.R353Q), GNB3 (c.825C>T), ICAM (p.R241G and p.K469E), IL6 (c.-174C>G), IL10 (c.-1082G>A), ITGB3 (p.L33P), MTHFR (c.677C>T), PPP3CA (p.Q318K), TGFB (p.L10P) and TNFA (c.-238G>A and c.-308G>A). SNPs were analyzed using the ABI 7500 and TaqMan probes and the ACE polymorphism was analyzed by agarose gel electrophoresis. Fisher's exact test was used to investigate a possible association between AR within one year after transplant (n=27) and a SNP for each gene in the SAS statistical software package. **Results:** The methylene tetrahydrofolate reductase (MTHFR) polymorphism (rs1801133) was the only SNP to give a suggestive association (P = 0.0612) with AR for all three genotypes. Inheritance of either the TT or TC genotype increased the risk of rejection (P = 0.0213; odds ratio of 2.3 (1.2-4.9)). The MTHFR SNP is associated with altered enzymatic activity. The T allele (frequency = 0.29) has been shown to reduce catalytic activity, resulting in an increase in serum homocysteine levels. Individuals homozygous for the C allele appear to be protected from AR whereas individuals either homozygous or heterozygous for the T allele were found to be at greater risk for acute rejection. **Conclusions:** Determining the genotypes of polymorphisms that affect transplant outcome may be important in the individualization of immunosuppression therapy. Randomized trials will need to be done to determine if individuals with either the TT or TC MTHFR genotypes would benefit from increased immunosuppression.

Association of *CD40L* IVS4+121 AG polymorphism with risk for coronary artery lesions in male Kawasaki disease patients. *Y. Onouchi*¹, *S. Onoue*¹, *T. Kawasaki*², *Y. Nakamura*^{3, 4}, *A. Hata*^{1, 5}. 1) Lab. Gastrointestinal Diseases, SNP Research Center, RIKEN, Yokohama, Japan; 2) Japan Kawasaki Disease Research Center, Tokyo, Japan; 3) Lab. Molecular Medicine, Human Genome Center, Institute of Medical Science, the Univ. of Tokyo, Tokyo, Japan; 4) Research Group for Personalized Medicine, SNP Research Center, RIKEN, Yokohama, Japan; 5) Dept. Public Health, Graduate School of Medicine, Chiba Univ., Chiba, Japan.

Kawasaki disease (KD) is an acute systemic vasculitis syndrome of infants and young children. Although KD is a basically self-limiting disease, 15 to 20% of untreated patients could suffer coronary artery aneurysms, a risk factor for life-threatening complications. Epidemiological findings suggest that genetic factors play a role in the pathogenesis of KD. To identify genetic factors, affected sib pair analysis has been performed. One of the identified peaks was located on Xq26 region. A recent report of elevated expression of CD40 ligand (CD40L), which maps to Xq26, during the acute phase KD and its relationship to the development of coronary artery lesions (CAL), prompted us to screen for polymorphism of *CD40L* and to study the association of the gene to KD. 22 single nucleotide polymorphisms (SNPs) were identified in 12.2kb of *CD40L* region. Among them, a newly identified SNP in intron 4 (IVS4+121 AG) is marginally over-represented in KD patients as compared to controls (109/602, 18.1% vs. 111/737, 15.1%). The difference was significant when male KD patients with CAL were compared to controls as a patient group (15/58, 25.9%, vs. 111/737, 15.1%, OR=2.0, 95%CI=1.07-3.66, $p=0.030$). Comparison between male patients with CAL and those without CAL also showed a significant difference (25.9% vs 14.1%; OR=2.1, 95%CI=1.02-4.21, $p=0.041$). Interestingly, this variation was extremely rare in a control Caucasian population (1/145, 0.7%). Our results suggest a role of CD40L in the pathogenesis of CAL and might explain the excess of males affected with KD.

Mutation Screening of 130 Candidate Genes Reveals a Complex Molecular Inheritance for Adult-Onset Primary Open Angle Glaucoma (POAG). *M. Sarfarazi*¹, *A. Child*², *L. Welsh*^{1,2}, *S. Suriyapperuma*¹, *S. Monemi*¹, *T. Rezaie*¹. 1) Molecular Ophthalmic Genetics Laboratory, University of Connecticut Health Center, Farmington, CT; 2) Cardiological Sciences, St Georges Hospital Medical School, London.

POAG is a group of ocular disorders with diverse clinical presentation, variable age of onset and considerable genetic heterogeneity. By using several genome scans we identified potential POAG loci on chromosomes 1, 2, 3, 4, 5, 6, 8, 10, 17 and 20. The purpose of this study was to identify the causative genes at every one of these loci. Potential candidate genes were selected based on their ocular expression and screened for mutation in their respective linked families. For each gene, affected subjects were screened by bi-directional sequencing and observed variations were evaluated bioinformatically against public databases. Co-segregation with POAG phenotype was evaluated by restriction enzyme analysis, SSCP or direct sequencing. Absence of POAG-causative mutation was confirmed in a group of normal control subjects from an ethnically matched population. We screened 130 genes from 10 different regions, including those mapping to the known GLC1A-GLC1E loci. For 2 intervals, every single known gene mapping to that region is now fully sequenced. Candidate genes had up to 80 exons and their cDNA sizes varied from 1 to 10-kb. Mutation screening revealed a large number of sequence alterations including many new SNPs, insertions, deletions and duplications. Identification of novel isoforms in a number of these genes added further complexity to molecular identity and verification of their role in POAG. Only 3 of the 130 genes screened were confirmed as POAG disease-causing genes. Confirmation, familial segregation, population screening and disease-causing nature of other observed variations are currently in progress. In conclusion, screening of a large number of candidate genes is required before a definite glaucoma-causing gene can be identified. This task is further complicated by presence of many naturally occurring polymorphisms within these genes. Supported By EY-09947, EY-014959, M01RR-06192 and IGA.

Association of NOS2A polymorphisms and pulmonary tuberculosis. *W.K. Scott^{1,2}, M.C. Levesque¹, L. Zhang², W.F. Hulme², N.N. Wall², C.A. Browning-Large², S.G. Patillo¹, A.W. Mosher¹, C.J. Pozsik³, J.R. Gilbert^{1,2}, J.B. Weinberg², C.D. Hamilton^{1,2}.* 1) Department of Medicine, Duke University Medical Center, Durham, NC; 2) Center for Human Genetics, Duke University Medical Center, Durham, NC; 3) South Carolina Department of Health and Environmental Control, Columbia, SC.

Tuberculosis (TB) is a significant cause of premature mortality worldwide. Approximately 10% of exposed individuals develop active pulmonary TB, suggesting that host defense factors, partly under genetic control, determine development of active disease. The NOS2A (inducible nitric oxide synthase) gene has been implicated in the development of mycobacterial infections in mouse models and is an attractive candidate gene for human TB susceptibility. We examined five polymorphisms in the promoter region of the NOS2A gene for association with TB in a family-based association study. Individuals reported to the North Carolina and South Carolina TB Control programs between 1992 and 2004 were eligible for the study. Individuals older than 14 years with culture-confirmed pulmonary TB and children younger than 14 years with culture- or clinically-confirmed TB were enrolled along with unaffected sibling or parental controls. Genotypes were obtained for 228 individuals from 104 families (68 African-American and 36 white). The TRANSMIT program (Clayton 1999) was used to analyze each polymorphism for association with TB in each ethnic group. Associations were assessed using the bootstrap empirical p-value provided by TRANSMIT. No polymorphisms were significantly associated with TB in the white subset ($p > 0.05$). However, the minor allele (4% frequency) of one polymorphism (-5091C) was associated with TB in African-Americans ($p=0.01$). These results indicate that at least one rare polymorphism of this gene may be over-transmitted to African-Americans with TB. However, given the low allele frequency of the variant, the robustness of this association is unclear. We will continue to follow-up by genotyping additional families for these and other polymorphisms in NOS2A.

Investigation of the dopamine D5 receptor gene in adult Attention Deficit Hyperactivity Disorder. *A. Squassina*¹, *U. Jain*², *V. De Luca*¹, *M. Krinsky*¹, *P. Muglia*¹, *J. Kennedy*¹. 1) Neuroscience section, Centre for Addiction and Mental Health, Department of Psychiatry, Clarke site, Toronto, Ontario, Canada; 2) Division of Child Psychiatry, Centre for Addiction and Mental Health, Toronto, Ontario, Canada.

Attention Deficit Hyperactivity Disorder (ADHD) is a common childhood disorder characterized by inattention, hyperactivity and impulsivity. It affects 3-5% of school-aged children and persists into adulthood in about 30% of cases. Twin, family and adoption studies have shown a large genetic component in the etiology of ADHD. Several lines of evidence indicate that the dopaminergic system play a role in the aetiology of ADHD. Numerous studies have also shown positive association among various polymorphisms at the dopaminergic system genes and ADHD. Previous investigations have examined the association between a dinucleotide (CA)_n repeat polymorphism, located in the cosmid containing the DRD5 gene, and ADHD. Some reports have shown a preferential transmission of the 148 bp allele ADHD children. In the present study we tested the association between ADHD and the CA repeat in our sample comprised of 110 families and 88 cases with matched controls. The E-TDT results did not show significant biased transmission of the 148 bp allele however, there was a trend for the over-transmission of this allele to ADHD offspring (T=42, NT= 30, $2 = 2.00$, $p=0.15$). An excessive non transmission of the 152 bp allele was observed suggesting a protective effect of this allele ($2 = 6.00$, $p=0.01$). The quantitative trait locus analysis, performed comparing the different genotype groups among the Brown and Wender scales score, did not detect any significant difference. Our study didnt show a strong effect for the CA repeat at the DRD5 locus in increasing susceptibility to ADHD. However, further analysis on a bigger sample are warranted.

Systematic screening of PLA2G7 gene for polymorphic variants and a preliminary association study in coronary artery disease patients. C. Terranova¹, M. Gomez-Lira¹, L. Provezza¹, N. Martinelli², C. Bozzini², M.R. D'Apice³, S. Gambardella³, G. Novelli³, P.F. Pignatti¹. 1) Department of Mother and Child, Biology and Genetics University of Verona, Verona, Italy; 2) Department of Clinical and Experimental Medicine, University of Verona, Verona, Italy; 3) Center of Excellence for Genomic Risk Assessment in Multifactorial and Complex Diseases, School of Medicine, Tor Vergata University of Rome, Rome, Italy.

Elevated levels of lipoprotein-associated phospholipase A2 (Lp-PLA2, or platelet-activating factor acetylhydrolase, PAF-AH) have been shown to be an independent risk factor for patients with angiographically proven coronary artery disease (Caslake et al 2000, Packard et al 2000). The entire coding region, the 3' flanking region, and 3,500bp of the 5'flanking region of the gene for Lp-PLA2 (PLA2G7) were systematically screened using DHPLC analysis in 100 Italian subjects with (CAD, 50 individuals) or without (CAD-free, 50 individuals) angiographically documented severe coronary atherosclerosis. Nine polymorphic variants were identified in these individuals: three known missense mutations in the coding region (Arg92His, Iso198Thr, and Val379Ala), a known deletion polymorphism (1190-20_23delGATT) in intron 11, two newly described highly polymorphic dinucleotide repeats (1134+31TGmAGn) in the 3 flanking region, and three SNPs in the 5 flanking region (-402T>C and 209C>G, -280T>C) one of them (-280T>C) newly described. No statistically significant difference in frequency the of alleles or genotypes of these PLA2G7 polymorphisms was observed between CAD and CAD-free individuals, suggesting that these sequence variants do not play an important role in susceptibility to coronary artery disease in this small study group. References: Caslake MJ, Packard CJ, Suckling KE, Holmes SD, et al (2000) Lipoprotein-associated phospholipase A2, platelet- activating factor acetylhydrolase: a potent new risk factor for coronary artery disease. *Atherosclerosis* 150; 413-419). Packard CJ, Denis SJ, O'Reilly, Muriel MD, et al (2000) Lipoprotein-associated phospholipase A2 as an independent predictor of coronary heart disease. *The New Eng J of Med* 343:1148-55).

Support for EKN1 as the susceptibility locus for reading disabilities and attention deficit hyperactivity disorder

on 15q21. *K. Wigg¹, J. Couto¹, Y. Feng¹, J. Crosbie², B. Anderson², T. Cate-Carter², R. Tannock², M. Lovett², T. Humphries², J.L. Kennedy³, A. Ickowicz², T. Pathare², W. Roberts², M. Malone², R. Schachar², C.L. Barr^{1,2}.* 1) Cell & Molecular Division, Toronto Western Hospital, Toronto, Ontario; 2) Brain and Behaviour Programme, The Hospital for Sick Children, Toronto, Ontario; 3) Neurogenetics Section, Centre for Addiction and Mental Health, Department of Psychiatry, University of Toronto, Toronto, Ontario, Canada.

Reading disabilities (RD) have been linked to a number of chromosomal regions including 15q. Recently a gene in the 15q region, EKN1, was identified via a translocation breakpoint in a family segregating with RD. Association studies in a small sample of families from Finland provided further support for this gene as a susceptibility locus for RD. In our sample of 148 families identified through a proband with reading difficulties, we found evidence for allelic association of polymorphisms in the EKN1 gene to the phenotype of RD identified as a categorical trait ($p=0.018$) and, using quantitative analysis, to reading related processes. There is considerable evidence from twin studies for shared genetic factors contributing to RD and attention-deficit hyperactivity disorder (ADHD), particularly inattention symptoms; therefore, we investigated the relationship of genetic markers in this gene to ADHD and the inattention and hyperactive/impulsive symptoms, in a sample of 186 nuclear families collected through a proband with the disorder. We found a trend for linkage to the ADHD phenotype as a categorical trait using the transmission disequilibrium test, and significant evidence for biased transmission of a haplotype containing this marker ($p=0.009$). Using quantitative analysis, we observed evidence for association of this haplotype to the inattention and hyperactive/impulsive symptoms, and a trend for association with the reading phenotypes of word identification and decoding. Our results provide preliminary support for the role of the EKN1 locus in RD and ADHD suggesting that this locus may be pleiotropic, contributing to both phenotypes.

Exclusion of TBX2 and TBX4 as candidate genes in MYP5-linked high myopia. *T.L. Young¹, P.C. Paluru¹, S. Nallasamy¹, W. Cole², E. Heon².* 1) Divisions of Ophthalmology and Genetics, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Ophthalmology and Vision Science Research Program, University Health Network, Toronto, Ontario, Canada.

TBX2 and TBX4 are T-box genes of the TBX2/3/4/5 subfamily of highly evolutionarily conserved putative transcription regulators that play critical roles in development. TBX2 and TBX3 are considered cognate genes, as are TBX4 and TBX5. TBX2 is known to be expressed in the human and mouse neural retina and mis-expression of TBX2 causes morphologic eye defects in *Xenopus*. TBX4, although primarily involved in hind limb development, may play a yet undiscovered role in eye development or in the regulation of TBX2 expression. Both TBX2 and TBX4 map within the high-grade myopia-5 locus (MYP5) located on chromosome 17q21-23. We screened individuals from the original MYP5 family for sequence variations in these genes. The genomic DNA from two affected individuals (proband spherical refractive error of -50.00 diopters in both eyes), two unaffected relatives, and one normal external control was screened by direct sequencing. Nine primer pairs spanning intron-exon boundaries and coding regions were designed for the 6-exon TBX2 gene. Similarly, 10 primer pairs were designed for the 8-exon TBX4 gene. Polymerase chain reaction products were sequenced and analyzed using standard fluorescent methods, and sequences were aligned with known database gene sequence for base pair change analysis. Sequencing of TBX2 amplicons revealed 10 polymorphisms, 6 of which were novel, and sequencing of TBX4 amplicons revealed 12 polymorphisms, 5 of which were novel. No polymorphism for either gene segregated with high myopia. TBX2 and TBX4 have been excluded as candidate genes for MYP5-associated high myopia.

Evidence for Gene Environment Interaction in HNF-3 Mediated MMP-7 Induction in Idiopathic Pulmonary Fibrosis. *Y. Zhang*¹, *A. Pardo*², *Y.P.P. Di*³, *B.S. Maher*⁴, *L. Wang*¹, *A.M.K. Choi*¹, *M. Selman*⁵, *N. Kaminski*¹. 1) Dept Medicine, Div PACCM, Univ Pittsburgh Sch Med, Pittsburgh, PA; 2) Facultad de Ciencias UNAM, Mexico DF, Mexico; 3) Dept Environmental and Occupational Health, Univ of Pittsburgh Sch public Health, Pittsburgh, PA; 4) Center Craniofacial and Dental Genetics, Univ Pittsburgh Sch Dental Med, Pittsburgh, PA; 5) Instituto Nacional de Enfermedades Respiratorias, Mexico DF, Mexico.

Idiopathic pulmonary fibrosis (IPF) is a devastating disease characterized by the expansion of fibroblasts/myofibroblasts, usually forming discrete subepithelial foci, and by the excessive accumulation of extracellular matrix which result in progressive and severe distortion of the pulmonary architecture. Although the etiology of IPF is unknown, a number of environmental exposures has been associated with elevated risks for IPF development. Particularly, a growing body of evidence strongly suggest that chronic exposure to cigarette smoke is a major risk factor to develop IPF. The genetic mechanisms that underlie the development of IPF and their association with environmental risk factors are unknown. Here we report evidence for gene-environment interaction in IPF based on our analysis of the matrix metalloprotease 7 (MMP-7) promoter single nucleotide polymorphisms, -181A/G and 153C/T. The 181A allele (homozygote or heterozygote) was significantly over-represented in Mexican smokers with IPF compared to normal Mexican smokers (95.7% vs 81.3%, $P = 0.0352$). The G to A transition resulted in a novel binding site for hepatocyte nuclear factor 3 (HNF-3). In-vitro, this transition led to allele specific HNF-3 binding to the MMP-7 promoter, and increased sensitivity of the MMP-7 promoter to HNF-3b. Interestingly, exposure to cigarette smoke extract induced expression of MMP-7 and HNF-3b. Our results suggest that the increased sensitivity of the polymorphic MMP-7 promoter to HNF-3b provides susceptibility to IPF in smokers carrying the 181A allele. In addition to being the first evidence for gene-environment interaction in IPF our results suggest a new general mechanism for smoking induced lung injury in humans.

SLE patients show low levels of CD4⁺CD25⁺CD45RO⁺ regulatory T cells which are extremely heritable and associated with the *CTLA4* gene. *M. Barreto*¹, *R. Ferreira*¹, *C. Fesel*¹, *M.F. Fontes*¹, *E. Santos*³, *M. Alves*³, *N. Cortez-Dias*³, *C. Pereira*², *B. Martins*², *R. Andreia*³, *J.F. Viana*³, *L. Mota-Vieira*⁴, *C. Vasconcelos*³, *C. Ferreira*³, *J. Demengeot*¹, *A.M. Vicente*¹. 1) Inst Gulbenkian Cincia, Oeiras, Portugal; 2) Instituto de Cincias Biomedicas Abel Salazar, Porto, Portugal; 3) Associacao dos Doentes com Lupus, Lisboa, Portugal; 4) Hospital Divino Esprito Santo, Ponta Delgada, Azorean Islands, Portugal.

Naturally occurring CD4⁺CD25⁺CD45RO⁺ regulatory T cells (Treg) play a key role in suppressing self-reactive T cells, contributing to the maintenance of immunologic self-tolerance and to autoimmune disease protection. With the purpose of investigating the involvement of these Treg in Systemic Lupus Erythematosus (SLE) pathogenesis, we have determined the relative numbers of CD4⁺CD25⁺CD45RO⁺ T cells in a population of 71 patients, 156 relatives and 193 healthy individuals. We found a significant difference in the distribution of the CD4⁺CD25⁺CD45RO⁺ T cell numbers among patients and controls ($H=37.49$, $p<0.0001$), with the patients showing significantly lower levels of Treg cells than healthy individuals. The numbers of Treg cells in SLE relatives showed an intermediate distribution between patients and controls and heritability for this trait was estimated as 85% ($p=0.0000002$). Given the high heritability of this trait, we searched for genetic factors determining the levels of Treg cells. Because cytotoxic T lymphocyte associated antigen 4 (CTLA-4) is a downregulator of the immune response and is constitutively expressed in regulatory T cells, we further investigated the involvement of the *CTLA4* gene in Treg levels. For this purpose we genotyped two polymorphic markers within the *CTLA4* gene. We found an association of a 3'UTR microsatellite and the levels of Treg cells ($H=19.320$ $p=0.023$). Individuals carrying a specific genotype, which had previously been found to increase risk for SLE, showed lower levels of these cells. These results suggest that *CTLA4* risk allele induces a reduced suppressive activity of these cells and consequently the proliferation of effectors, leading to the development of autoimmune disease.

Genetic Characterization of the CD40 P227A Polymorphism: A Gain-of-Function Polymorphism Associated with Human SLE. *P.M. Gaffney¹, A.C. Grammer², L. He², R.M. Plenge³, R.R. Graham³, W.O. Ortmann¹, P.K. Gregersen⁴, D.M. Altshuler³, P.E. Lipsky², T.W. Behrens¹.* 1) Department of Medicine, University of Minnesota School of Medicine, Minneapolis, MN; 2) Autoimmunity Branch, NIH/NIAMS, Bethesda, MD; 3) Broad Institute, MIT/Whitehead Institute for Biomedical Research, Boston, MA; 4) North Shore-Long Island Jewish Research Institute, New York, NY.

CD40 is a surface receptor on B lymphocytes and dendritic cells that plays a critical role in immunity against pathogens. CD40 is located within a region of genetic linkage to SLE on chromosome 20q13.1. To further investigate the role of CD40 in SLE we sequenced all coding exons and intron/exon boundaries and discovered a novel missense single nucleotide polymorphism (SNP) in the cytoplasmic domain of CD40, which results in a proline-to-alanine mutation at amino acid 227 (P227A). In P227A carrier families with SLE, P227A was preferentially transmitted to affected offspring (22 of 27, 81%, $p=0.0038$) suggesting a role for P227A in the pathophysiology of SLE. Our data also suggested an increased prevalence of P227A in Hispanic families (5 of 15, 30%) compared with Caucasian families (6 of 196, 3%). To investigate this, we genotyped P227A in unaffected Caucasian, African American and Hispanic control populations from the United States and the CEPH Diversity Panel, comprised of DNA from 51 indigenous populations in 60 geographic locations throughout the world. These analyses confirmed the increased allele frequency of P227A in Hispanics (3.6%) compared to Caucasians (0.41%) or African Americans (1.0%) in the United States. The results in the CEPH Diversity Panel revealed striking allele frequencies exclusively in populations from South and Central America (range 7.1%-46%) suggesting the influence of selective pressure. Importantly, functional studies show that the CD40 P227A receptor constitutively binds the adaptor protein TRAF2, which in turn leads to signaling through the mutant receptor in the absence of ligand-induced receptor aggregation. Thus, CD40-P227A is a common genetic variant with a gain-of-function phenotype that predisposes to SLE.

Mannose-binding lectin (MBL) genetic susceptibility for Alzheimers disease. *L. Nuytinck*¹, *M. Palmer*², *S. Schraen-Maschke*³, *M. Duhamel*¹, *M. Van Thielen*¹, *T. Van de Castele*¹, *A. Johansson*², *A. Delacourte*³, *B. Sablonniere*³, *K. Blennow*², *E. Vanmechelen*¹. 1) Innogenetics NV, Gent, Belgium; 2) Gteborg University, Sweden; 3) INSERM U422, Lille, France.

In sporadic Alzheimers disease (AD) it has been estimated that apart from ApoE4, a number of additional loci can determine AD genetic risk. As evidence has accumulated indicating a chronic inflammatory reaction in areas of the brain affected by AD, we explored the possible role of innate immunity in AD pathogenesis. As a first target, the gene encoding mannose-binding lectin (MBL) was studied due to its key role in innate immunity, and in view of reported decreased MBL levels in the cerebrospinal fluid of AD patients compared to controls. The main functions of MBL are the activation of the complement system through the lectin pathway and the modulation of inflammation. Six common *MBL2* DNA variants localized in the promotor, 5'UTR and exon 1 sequences, are known to result in considerable variations in the quantity and functionality of MBL in serum. In order to investigate the genetic susceptibility of common *MBL2* genotypes for the development of AD, a case-control study was performed consisting of 523 AD patients and 285 age-matched controls from Swedish and French centers. Genotyping was performed using the INNO-LiPA MBL2 and the INNO-LiPA ApoE assays (Innogenetics, Gent, Belgium). Using a logistic regression approach, it was tested if disease incidence changed depending on the presence of the 'promotor deficient' *MBL2*-haplotype LXPA, and the pooled 'non-functional' *MBL2*-haplotypes HYPD, LYPB and LYQC, as compared with the pooled *MBL2*-haplotypes HYPA, LYPA and LYQA. AD-incidence tended to increase with the presence of LXPA ($p=0.0657$, OR 1.34 [95% CI 0.98-1.84]) and significantly decreased with the presence of the non-functional haplotypes ($p=0.0304$, OR 0.72 [95% CI 0.53-0.97]). No significant interactions between ApoE and the *MBL2*-(pooled) haplotype(s) were observed ($p0.45$). Our results indicate that genetic variation in the *MBL2* gene could be associated with the development of AD. This may have important implications for the diagnosis and treatment of this disease.

Epigenetic alteration of Angelman syndrome gene (*UBE3A*) in autism brains. Y. Jiang, Q. Liu, L. Dai, A.L. Beaudet. Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

Numerous cytogenetic studies have indicated that chromosome 15q11-q13 and genomic imprinting may play an important role in the genetic etiology of autism. Maternally inherited interstitial duplications of 15q11-q13 and an isodicentric chromosome 15 of maternal origin are the two most common cytogenetic abnormalities found in autism patients. Two disorders of genomic imprinting, Prader-Willi syndrome (PWS) and Angelman syndrome (AS), involve abnormalities of the 15q11-q13 region. Maternal deficiency of chromosome 15q11-q13 and mutations in *UBE3A*, a brain-specific and maternally expressed ubiquitin protein ligase, are the major causes of AS. There are significant overlapping clinical features between AS and autism. We tested a hypothesis that *UBE3A* is a candidate gene from 15q11-q13 and that epigenetic alteration of *UBE3A* contributes to autism susceptibility. We have analyzed DNA methylation of CpG islands at the 5' ends of *SNRPN* and *UBE3A*, as well as a newly identified tissue-specific DNA methylation site at the 3' end of *UBE3A* in autopsy brain tissues and cell lines from autism patients. We have identified abnormal DNA methylation at the 5' *UBE3A* CpG island in 1 out of 17 brain tissues. The 5' *UBE3A* CpG island is completely unmethylated in 60 normal controls but became partially methylated in one autism brain. In addition, we also found a significant reduction of *UBE3A* protein in 7 out of 17 autism brain tissues by Western blot analysis. These findings are in press (Am J Med Genet). In subsequent work, abnormal DNA methylation at the 3' tissue-specific DNA methylation site was also identified in 1 out of 43 cultured lymphoblast cell lines from autism patients. Sequence alterations in the CpG island/promoter region of *UBE3A* were also identified in autism patients and the functional significance of these alterations is being investigated in an *in vitro* assay. The available data suggest that dysregulation of *UBE3A* gene expression in the brain may contribute to autism susceptibility and that epigenetic alterations may play an important role in autism.

Lack of association between VIPR2 gene variants and Autism Spectrum Disorders (ASDs) in Case-Control and Family Based Association Studies. X. Liu^{1,3}, J.J.A. Holden^{1,2,3}, ASD-CARC⁴. 1) Dept of Psychiatry; 2) Dept of Physiology, Queens University, Kingston, ON, Canada; 3) Autism Research Program, Ongwanada Resource Centre, Kingston, ON, Canada; 4) www.autismresearch.ca.

The etiology of ASDs is unknown, but widely accepted as resulting from the interaction of multiple genes and environmental factors. Vasoactive Intestinal Peptide (VIP) is a peptide hormone expressed in both the gastrointestinal tract and the brain. It has been shown to reduce autistic symptoms in children infused with secretin for the assessment of gastrointestinal complaints. A recent report of elevated levels of VIP in blood samples from newborns later diagnosed with autism has spurred an interest in this gene and its receptor in the etiology of ASD. Of genes involved in the VIP pathway, VIPR2 gene is of particular interest because of its abundant expression in the embryonic and postnatal brain and its location at 7q36, near the distal portion of the broad autism linkage peak found in several genome scans. A recent study with a small sample size suggested that the VIPR2 gene might play a role in susceptibility to autism. To determine whether these findings could be confirmed, we genotyped the same two polymorphisms in 180 multiplex families, 58 simplex families and more than 250 control samples. We performed both a case-control study and examined allele and haplotype transmission using FBAT, and did a genotype-phenotype association analysis based on the available ADI-R data. Our results demonstrated no deviation from the null hypothesis of no association of VIPR2 variants with autism susceptibility. We have also separately examined transmissions for paternal versus maternal alleles, transmission to affected boys versus girls, and subgrouped the multiplex families into groups with exclusively males and those with at least one affected girl. None of these subsets revealed significant deviation from the null expectation. Our interpretation of these findings is that it is unlikely that the VIPR2 gene plays a significant role in the genetic predisposition to autism. (Supported by CIHR and OMHF).

Genetic association studies in gallstone disease in North India. *B. Mittal, M. Dixit.* Dept of Genetics, Sanjay Gandhi Postgrad Inst, Lucknow, UP, India.

Background: Gallstone disease is common in western countries as well as in India. It is a multifactorial disease. The increased incidence and virulence in certain populations, high concordance in monozygotic twins, and clustering of gallstone disease in families suggest the possibility of a genetic predisposition to the disease. A prerequisite for the development of cholesterol gallstone is lithogenic bile, which is often the result of enhanced cholesterol synthesis or a reduced bile acid pool size, or both. Nucleation of cholesterol monohydrate crystals is an initial and essential step in the process of gallstone formation. In humans, several genetically derived phenotypes are responsible for variation in lipoprotein and lipoprotein metabolizing enzyme types, which in turn may affect the concentration of cholesterol in bile.

Aim: Present study was undertaken to reveal the association of apolipoprotein B XbaI, apolipoprotein HhaI, LRPAP1 intron 6 insertion (I)/ deletion (D), lipoprotein lipase HindIII, and LDL receptor AvaII polymorphisms with gallstone disease in North India.

Materials and Methods: Blood samples were collected from 100 gallstone patients and 200 ultrasonographically confirmed gallstone free healthy subjects. Genotyping was done by PCR-RFLP followed by agarose / polyacrylamide gel electrophoresis. In case of LRPAP1 insertion/ deletion polymorphism PCR products were electrophoresed directly.

Result: There were no significant differences in allele frequencies of LDLR AvaII polymorphism in patient and control group. In contrast to other studies no association of apolipoprotein 4 allele was found with gallstone disease. In LRPAP1, II genotype and I allele were significantly higher [(p=0.003) and (p= 0.013) respectively] in patients than in controls. X+ allele of Apolipoprotein B was significantly lower (p= 0.000) in patients as compared to controls. Frequency of L1L2 genotype (LPL HindIII polymorphism) was also significantly lower (p= 0.000) in patients than in controls.

Conclusion: Presence of I allele of LRPAP1 in homozygous or heterozygous form and absence of X+ allele and L1L2 genotype may confer higher risk for development of gallstones.

Serotonin System Genes and Childhood-Onset Mood Disorder (COMD). *S.A. Shaikh¹, J. Strauss¹, N. King¹, A. Vetro², E. Kiss², Z. Tamas³, C. George⁴, M. Kovacs⁴, C. Barr⁵, J. Kennedy¹.* 1) Neurogenetics, Centre for Addiction and Mental Health, Toronto, Ontario, Canada; 2) Szeged University Medical Faculty, Department for Child & Adolescent Psychiatry; 3) Vadaskert Hospital, Budapest, Hungary; 4) Western Psychiatric Institute and Clinic, UPMC Health System, Pittsburgh, USA; 5) University Health Network-Toronto Western Research Institute, Department of Psychiatry, University of Toronto.

The serotonin system has been extensively studied and implicated in various neuropsychiatric disorders including mood disorders. We have investigated two of the receptors, 5HT1B and 5HT2A, and the serotonin transporter (SLC6A4). We have collected two independent samples of childhood-onset mood disorder (COMD), one in Pittsburgh consisting of 150 case-control pairs and the other in Hungary consisting of 218 small families. In our case-control analysis, we have found no association between childhood-onset mood disorder and the G861C polymorphism of 5HT1B and the T102C polymorphism of 5HT2A by allele frequencies ($\chi^2 = 0.01$; $p = 0.91$ and $\chi^2 = 1.35$; $p = 0.24$, respectively), or genotype frequencies ($\chi^2 = 0.37$; $p = 0.83$ and $\chi^2 = 1.63$; $p = 0.43$, respectively). Analysis of the 17 bp variable number tandem repeat in the second intron of the serotonin transporter gene, 5HTTVNTR, was not significant in the Pittsburgh sample (allele frequencies $\chi^2 = 3.10$; $p = 0.21$; genotypes $\chi^2 = 4.20$; $p = 0.32$).

Analysis of our Hungarian COMD families by transmission disequilibrium test (TDT) showed similar results to our case-control study with no association with 5HT1B (T102C) and 5HT2A (G861C) polymorphisms. However, analysis of the VNTR polymorphism showed preferential transmission of the 12 repeat ($\chi^2 = 4.72$; $p = 0.03$), and combining the Pittsburgh and Hungary samples for the 5HTT VNTR produced a Z score of 2.71, $p = 0.003$. Overall our examination of the serotonin 1B and 2A is mostly negative, however we found evidence that the 12 repeat allele of the 5HTTVNTR polymorphism may play a significant role in the pathogenesis of COMD. Further examination of the serotonin system, particularly the 5-HTTLPR polymorphism in a larger sample, may be warranted.

Association between the C-45T polymorphism of the Cholecystokinin Gene and Panic Disorder. S.

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Panic disorder (PD), affecting 1-3% of the population, is classified as an anxiety disorder, where individuals experience sudden onset of panic attacks and fear of future attacks. The cholecystokinin system has been previously implicated in PD, and is suggested to play a neurobiological role through its interaction with dopamine and its influence on dopamine-induced behaviour. We genotyped, using TaqMan assays and the ABI7000, the C-45T polymorphism in the Sp1 binding site of the promoter region of the CCK peptide gene located on chromosome 3pter-q12, along with four other polymorphisms found within intronic regions of the gene. We first investigated the five CCK polymorphisms in 297 individuals from 94 small nuclear families and found the C-45T promoter polymorphism to be significantly associated with PD. Our transmission disequilibrium test (TDT and s-TDT) analysing this gene in the families yielded a chi-square of 8.048 ($p=0.005$). In a second sample of case-control pairs using the same polymorphisms, individuals were matched for sex, age, and ethnicity. There was a significant difference in the distribution of alleles of the C-45T polymorphism between the 101 case-control matched pairs ($p=0.04$). The other four polymorphisms did not show any association with panic disorder. Our findings support the involvement of CCK gene in panic disorder, but further analysis of other polymorphisms in this gene or other genes of the CCK system is warranted.

Interaction of a Region on Chromosome 11q23 and the HLA-DPB1 Locus Influences Susceptibility to Ankylosing Spondylitis (AS). *J. Reveille*¹, *G. Zhang*², *M.H. Weisman*³, *J. Bruckel*⁴, *H.R. Schumacher*⁵, *M.A. Khan*⁶, *R.D. Inman*⁷, *W.P. Maksymowych*⁸, *T.M. Martin*⁹, *M. Stone*⁷, *J.T. Rosenbaum*⁹, *L. Jin*². 1) Div Rheumatology, Univ Texas Medical Sch, Houston, TX; 2) University of Cincinnati, Cincinnati, OH; 3) Cedars-Sinai Medical Center, Los Angeles, CA; 4) Spondylitis Association of America, Sherman Oaks, CA; 5) The University of Pennsylvania, Philadelphia, PA; 6) Case Western Reserve University, Cleveland, OH; 7) The University of Toronto, Toronto, ON, Canada; 8) The University of Alberta, Edmonton, AB, Canada; 9) Oregon Health & Science University, Portland, OR.

To study the interaction of chromosomal regions previously identified in a genomewide scan of familial AS susceptibility with genes of the major histocompatibility complex (MHC) and had available radiographs or reports thereof. All patients met modified New York Criteria for AS (1984). MHC genotypes were determined by oligotyping with high resolution DRB1 typing by sequence analysis. Interaction between chromosomal regions (with markers with NPL score > 1.5 identified in the genomewide linkage analysis) and MHC was studied by performing conditional analyses. The weighting of each family was based on their evidence for linkage at these markers. Two weighting schemes were used to model a positive (for gene-gene interaction) and a negative (for heterogeneity gene-gene interaction) relationship between loci on different chromosomes, respectively. Based on this conditioning scheme, the conditional allele-sharing model LOD scores were calculated by ASM computer program. 244 affected sib pairs from 180 pedigrees were analyzed, including 602 family members, of whom 424 had AS. An additional 39 pedigrees containing 129 family members (48 with AS) were included, of whom two had affected sib pairs but the other sib was not available for study and 37 were trio families (one affected proband with parents available). A region on chromosome 11 (11q23) showed statistically significant interaction with MHC.

Resegregating behaviors in the Silver Fox. A model system for mapping sociability. G.M. Acland¹, H.N. Erb¹, K. Chase², K.G. Lark², G.D. Aguirre³, L. Trut⁴, I. Oskina⁴, A. Kharlamova⁴, A.V. Kukekova¹. 1) College of Veterinary Medicine, Cornell Univ, Ithaca, NY; 2) Univ of Utah, Salt Lake City, Utah; 3) School of Veterinary Medicine, Univ of Pennsylvania, Philadelphia, PA; 4) Institute of Cytology and Genetics, Novosibirsk, Russia.

Silver Fox (*Vulpes vulpes*) strains have been bred to exhibit tame behavior like pet dogs (*Canis familiaris*), or wildtype aggressive behavior. These studies recapitulate the process in which dogs were domesticated from the Grey Wolf (*Canis lupus*), but were accomplished in 35 generations under carefully recorded observation. The resulting differences between tame and wildtype fox strains provide clear evidence for heritability of traits similar, in one case, to the normal sociability of human children, and in the other to autism and anxiety disorders. Informative pedigrees have now been developed by crossing tame and wildtype foxes, and backcrossing F1 individuals to mates from the tame population. A newly refined method of measuring behavior in these populations identified 50 binarily scoreable observations that nonredundantly and reliably distinguished behaviors of the reference populations (Tame, Wildtype, F1). Principal-components analysis was used to dissect out independent elements underlying these behaviors. PC1 accounted for 47.3% of total variance in measured traits; PC2 for 6.4 %. This system clearly discriminated tame foxes from wildtype foxes. F1 foxes yield intermediate values that extend into the ranges of both the tame and wildtype foxes. Behavioral scores PC1 and PC2 of backcross foxes cluster bimodally with one subgroup well within the tame range, and a second with values similar to those of F1 and wildtype foxes. Heritability and power analyses demonstrate strong support for a mapping project in progress to map QTLs underlying these resegregating traits. This project, utilizing microsatellite markers adapted from the canine genome and validated in the Silver Fox, holds considerable promise for identification of loci potentially important for both normal human behavior, and recognized hereditary disorders of human sociability.

Genome-wide linkage scan for the metabolic syndrome reveals a major quantitative trait locus on chromosome 15q: The Quebec Family Study. *Y. Bosse*^{1,2}, *J.-P. Després*^{2,3}, *Y.C. Chagnon*⁴, *T. Rice*⁵, *D.C. Rao*⁵, *C. Bouchard*⁶, *L. Pérusse*⁷, *M.-C. Vohl*^{1,2}. 1) Lipid Research Ctr, Laval Univ, Ste-Foy, PQ, Canada; 2) Department of Food Science and Nutrition, Laval Univ, Quebec, PQ, Canada; 3) The Quebec Heart Institute, Quebec, PQ, Canada; 4) Laval Univ Robert-Giffard Research Ctr, Quebec, PQ, Canada; 5) Division of Biostatistics, Washington Univ School of Medicine, St. Louis, MO, USA; 6) Pennington Biomedical Research Ctr, Baton Rouge, LA, USA; 7) Division of Kinesiology, Department of Social and Preventive Medicine, Laval Univ, Quebec, PQ, Canada.

The metabolic syndrome clusters multiple cardiovascular risk factors under one disorder entity. An autosomal genome-wide linkage scan was performed on 707 participants from 264 nuclear families of the Quebec Family Study in order to identify chromosomal regions harbouring genes predisposing to the metabolic syndrome. Maximum likelihood-based factor analysis was used to generate factors underlying the correlation structure among waist circumference, body mass index, plasma insulin and glucose levels, triglyceride and HDL-cholesterol levels, as well as systolic and diastolic blood pressure. Three major factors were identified and interpreted as metabolic syndrome, blood pressure and blood lipids. The metabolic syndrome factor had high factor loadings (0.4) for the eight metabolic syndrome-related phenotypes and explained 76.5% of the standardized variance. Family lines accounted for 45.6% of the metabolic syndrome factor variance. The most significant genome-wide variance component linkage with this composite factor was found on chromosome 15q, with a LOD score of 3.15 at 86 cM. Suggestive evidence of linkage (LOD 1.75) was also observed on chromosomes 1p, 3p, 3q, 6q, 7p, 19q, and 21q. These quantitative trait loci should be further explored as they may harbour genes that contribute to the underlying correlation structure among the phenotypes defining the metabolic syndrome.

Association of PTPN1 with coronary artery calcification in the Diabetes Heart Study. *K.P. Burdon^{1,2}, J.L. Bento^{1,2}, N.D. Palmer^{1,2}, C.E. Langefeld³, J.K. Campbell³, S.R. Beck³, J.J. Carr⁴, D.M. Herrington⁵, B.I. Freedman⁵, S.R. Rich³, D.W. Bowden^{1,2,5}.* 1) Center for Human Genomics; 2) Biochemistry; 3) Public Health Sciences; 4) Radiology; 5) Internal Medicine, Wake Forest University School of Medicine, Winston-Salem, NC.

The protein tyrosine phosphatase, non-receptor type 1 gene (PTPN1) encodes the PTP1B protein, a ubiquitously expressed tyrosine phosphatase involved in several pathways, including negative regulation of both insulin and leptin signaling as well as regulation of integrin and cadherin function. We have observed significant associations between PTPN1 SNPs and both diabetes and measures of insulin resistance in Caucasian and Hispanic American populations. In the Diabetes Heart Study (DHS), we have investigated the association of PTPN1 with diabetic cardiovascular disease in 706 individuals from 267 Caucasian families. Phenotypes studied include carotid intima-media thickness (IMT), coronary (CAC) and carotid (CarAC) artery calcification and lipid measures. A total of 23 PTPN1 SNPs were genotyped. A single (~88 kb) block of linkage disequilibrium (LD) was detected encompassing the entire coding region of PTPN1. To test for association, GEE1 modeling was used, with age, gender, diabetes status and smoking status as covariates. The majority of SNPs within the LD block were found to be associated with CAC (p-values ranging from <0.0001 to 0.066). Haplotypes were computed with the EM algorithm for a subset of 8 SNPs. The most frequent haplotype (frequency = 0.41) was found to contribute to increased calcium scores with a p-value of 0.039 under an additive model. This haplotype was also found to confer risk for diabetes and insulin resistance in previous studies. A second haplotype (frequency = 0.09) differed from the risk haplotype at 5 of the 8 loci and was found to contribute to decreased calcium scores also under an additive model (p = 0.027). No consistent association was detected with IMT, CarAC, or lipid measures. These results suggest that the PTPN1 locus contributes to arterial calcification and diabetic cardiovascular disease.

The interferon gamma (IFNG) gene and its interaction with smoking are associated with lung function in smokers. *K. Burkett¹, J. He¹, J.E. Connett², N.R. Anthonisen³, P.D. Paré¹, A.J. Sandford¹.* 1) James Hogg iCAPTURE Centre, UBC, St Paul's Hospital, Vancouver, BC, Canada; 2) Division of Biostatistics, School of Public Health, University of Minnesota; 3) Faculty of Medicine, University of Manitoba.

It has been suggested that interactions between genetic factors and environmental determinants such as smoking may lead to better understanding of the pathogenesis of chronic obstructive pulmonary disease (COPD), which is diagnosed mainly based on lung function. We hypothesized that the IFNG gene and its interaction with smoking are associated with lung function in smokers in the Lung Health Study (LHS). We studied 4 single nucleotide polymorphisms (SNPs) in IFNG in 1061 Non-Hispanic whites with the lowest (n = 530, FEV1 % predicted = 62.6 0.1) and the highest (n = 531, FEV1% predicted = 91.8 0.1) lung function at the beginning of the LHS. The four SNPs are rs2069707, rs1861493, rs2069718 and rs2069727. The associations were analyzed by chi-square tests and logistic regression to adjust for potential confounding factors including age, sex, research center and pack - years. The rs2069727 SNP, which is in perfect linkage disequilibrium with a polymorphism affecting IFNG expression level, was associated with low lung function. The frequencies of the higher IFNG expression GG genotype were 26.1% and 18.0% respectively in low and high lung function groups, which was significantly different before and after adjustment for confounding factors (unadjusted OR = 1.6, 95% CI 1.2 2.2, p = 0.002; adjusted OR = 1.5, 95% CI = 1.1 2.1, p = 0.009). There was a significant gene and smoking interaction with p = 0.001 for the interaction term. The ORs decreased significantly as pack-years increased; the ORs (95% CIs) were 3.3 (1.7-6.3) in smokers with 20 pack years, 1.5 (1.0-2.1) in smokers with 40 pack years and 0.8 (0.5-1.3) in smokers with 60 pack-years. There were no associations between the other three SNPs and lung function. In conclusion, cigarette smoking modifies the association between IFNG SNP and lung function in smokers. Supported by the NHLBI Lung Health Study and the Canadian Institutes of Health Research.

Mutation Analysis of DARPP-32 as a Candidate Gene for Schizophrenia. *C.-H. Chen^{1, 2}, C.-H. Li¹, H.-M. Liao¹.* 1) Dept Psychiatry, Tzu-Chi General Hosp, Hualien City, 970 Taiwan; 2) Institute of Human Genetics, Hualien City, 970 Taiwan.

Background: Dopamine- and cAMP-regulated phosphoprotein of relative molecular mass 32 kD (DARPP-32) plays a pivotal role in mediating the signal transduction of many neurotransmitters and neuromodulators in brain that are implicated in the pathogenesis of mental disorders. A recent study demonstrated significant reduction of DARPP-32 protein in the dorsolateral prefrontal cortex (DLPFC) of patients with schizophrenia when compared to controls, suggesting that DARPP-32 may be involved in the pathophysiology of schizophrenia. In view of the high heritability of schizophrenia, we hypothesized that DARPP-32 might be a plausible candidate gene for conferring schizophrenia susceptibility. Methods: We directly sequenced the genomic DNA from patients with schizophrenia to search for mutations in the exon and core promoter regions of the DARPP-32 gene and conducted a case-control association study. Results: Five molecular variants were identified, including a C-to-G substitution at the core promoter that obliterated an AP-2 transcription factor binding site; a G deletion at untranslated exon 2; a G-to-A and an A-to-G substitutions at intron 2 and intron 6, respectively; and a three-base pair deletion of AGA at exon 6 that resulted in a deletion of a glutamate at codon 135. However, no differences of allele or genotype frequencies of these variants between patients and controls were noted. Conclusions: Our data indicate that the DARPP-32 gene may not be a major susceptibility gene for schizophrenia, and the reduced DARPP-32 protein in the DLPFC of patients with schizophrenia is unlikely due to the mutations of the DARPP-32 gene.

Genetics of Gene Expression Variation in Radiation Response. *C. Correa*¹, *V.G. Cheung*². 1) School of Medicine, Univ. of Pennsylvania, Philadelphia, PA; 2) Depts of Pediatrics & Genetics, Univ. of Pennsylvania, The Children's Hospital of Philadelphia, PA. 19104, USA.

Humans are exposed to ionizing radiation (IR) through the environment and in medical settings. Much variation in IR response has been observed clinically among individuals. However, the genetic basis of this variation remains largely unknown. This is partly due to the lack of phenotypes that can be precisely measured and the difficulty of collecting family material for genetic studies. In this project, we are identifying the genetic contribution of variation in IR response by using the expression level of genes as phenotypes in lymphoblastoid cells lines from unrelated individuals and genetically identical monozygotic twins.

The focus of our study is a set of known IR responsive genes. First, we measured their expression levels at baseline and at two time-points post irradiation among unrelated individuals. We found extensive variation in the expression levels of these genes at baseline and in response to radiation. The extent of variation is greater after radiation. Second, to determine if there is a genetic component to the variation in IR-induced expression phenotypes, we measured the expression levels among 10 sets of genetically-identical monozygotic twins and compared the variance among twin pairs with the variance within twin pairs by means of the intraclass correlation coefficient (ICC). The ICC is the highest for three genes, PPM1D, FDXR and CDKN1A (ICC > 0.80). The induction of these genes is significantly ($P < 0.001$) greater among twin pairs than within twin pairs supporting that there is a genetic component to the variation in the expression levels of these genes. Further analysis shows that the distribution of the radio-response of ferredoxin reductase (FDXR), as measured by area-under-the curve for its expression level at baseline and post-IR, is distributed bimodally.

In this presentation, we will present our findings and describe our approach to dissect the genetic basis of radiation response. Similar study design can be applied to study the genetic architecture of other complex human quantitative phenotypes.

Bioinformatic assembly of the *GABRG3* gene and its role in autism spectrum disorder. *S. Curran, M. Kasahara, J. Powell, P. Bolton.* Psychological Medicine, Inst Psychiatry, London, United Kingdom.

Autism is a neurodevelopmental disorder with a significant genetic component of a complex nature. Genomic screens have demonstrated suggestive evidence for linkage to proximal chromosome 15q close to the cluster of gamma (γ)-aminobutyric acid receptor A (GABAA) subunit genes (*GABRB3*, *GABRA5* and *GABRG3*) and preliminary association studies support this. Cytogenetic abnormalities in this region have been described in several individuals with autism. The proximal portion of chromosome 15q is a complex region of the genome, containing an imprinting centre, numerous repetitive elements and duplicons. Developmental studies suggest that GABA plays an important role in neurogenesis. The subunits that make up the GABAA receptors have been shown to change during development, in particular the 3-subunit, which is present in embryonic cortex and spinal cord but not in significant amounts in adult tissues. Such transient expression of embryonic receptor types that respond to developmental signals rather than synaptic transmission may be important in neurodevelopmental disorders such as autism. The current assembly of the human genome (July 2003) for the chromosome 15 region that includes *GABRG3* is incomplete, only part of the gene is present. We have therefore attempted to assemble the complete sequence. Genome databases were searched for sequences highly homologous to *GABRG3* exonic sequences using the program *blastn* and a number of sequenced BAC and fosmid clones identified. Further searches with non repetitive sequences from the ends of these clones were used to identify additional sequences from the region. When manual assembly of these sequences was attempted, it was apparent that there is a large duplicated segment flanking exons 5 and 6. Similar low copy repeat elements can be associated with non homologous recombinational events leading to deletions and duplications. We are using a quantitative PCR dosage assay to examine copy number in this region in 145 well characterised individuals with autism spectrum disorders and their parents.

Association studies of schizophrenia with the ionotropic and metabotropic glutamate receptor genes. Y.

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Schizophrenia is a devastating neuropsychiatric disorder with a life time prevalence of about 1% of the world population. Since the glutamatergic dysfunction hypothesis was proposed for the pathogenesis of schizophrenia, we have been conducting a systematic study of associations between glutamate receptor (GluR) genes and schizophrenia. We report here genotype and haplotype association studies on two GluR genes, the NMDA receptor subunit NR2D gene, *GRIN2D*, and the metabotropic glutamate receptor mGluR5 gene, *GRM5*. [1]*GRIN2D*: We identified 13 SNPs by direct sequencing of the entire exons and the 5' upstream regions of the gene. We genotyped 200+ Japanese case-control pairs for 7 common SNPs selected from the 13 SNPs, with the average intervals, 8.35 kb. We observed no significant associations of any of the 7 SNPs with schizophrenia. However, we observed highly significant haplotype associations with the disease in 3 SNP-pairs out of all possible 21 pairs ($p = 1.09 \times 10^{-6}$, $p_{\text{corr}} = 2.29 \times 10^{-5}$, at the lowest). [2]*GRM5*: We genotyped 100 Japanese case-control pairs for 14 known SNPs of the gene with the average intervals, 42.4 kb. We observed no significant associations of any of the 14 SNPs with schizophrenia. However, we observed highly significant haplotype associations with the disease in one pair of the SNPs out of all possible 91 pairs ($p = 3.37 \times 10^{-4}$, $p_{\text{corr}} = 3.07 \times 10^{-2}$) We confirmed this significant association by expanding the sample size to 200+ ($p = 6.34 \times 10^{-5}$, $p_{\text{corr}} = 5.77 \times 10^{-3}$). We conclude that susceptibility loci for schizophrenia are probably situated within or very close to the *GRIN2D* and *GRM5* regions in Japanese.

Systematic mutation screening of protein phosphatase 1 catalytic subunit(PPP1CA) at 11q13.3 as a candidate gene for schizophrenia. *T.-W. Hung*¹, *C.-H. Chen*^{1,2,3}. 1) Institute of Human Genetics, Tzu-Chi University, Hualien, 970 Taiwan; 2) Department of Psychiatry, Tzu-Chi University Hualien, 970 Taiwan; 3) Tzu-Chi General Hospital, Hualien, 970 Taiwan.

Background: Dopamine- and cAMP-regulated phosphoprotein of relative molecular mass 32 kD (DARPP-32) was suggested to be involved in the pathogenesis of schizophrenia. Phosphorylated DARPP-32 is an inhibitor of protein phosphatase-1 (PP1). The PP1 is a major serine/threonine protein phosphatase in eukaryotic cells that regulates phosphorylation and activity of numerous physiologically important substrates, including neurotransmitter receptors, voltage-gated ion channels, ion pumps, and transcription factors. Previous studies reported that one of the catalytic subunit of PP1 is associated with schizophrenia, suggesting that PP1 is also involved in the pathogenesis of schizophrenia. The purpose of this study is to examine whether another one of the catalytic subunits of PP1-PPP1CA is also associated with schizophrenia. Methods: To test this hypothesis, we systematically screened the protein-coding sequences and promoter region of the PPP1CA gene in a sample of schizophrenic patients using direct sequencing. Results: In 48 patients we found six polymorphisms including two G to C substitution at the core promoter region (-132G/C, -63G/C); a C to G substitution at intron 3 (IVS3-8C/G); a A to G substitution at intron 4 (IVS4+48A/G); a C to T substitution at intron 5 (IVS5-10C/T) and a G to A substitution at 3' UTR (c.1113G/A), respectively. To determine their association with schizophrenia, we currently carrying out case-control study with a larger sample size.

Family-based Association Analysis of the Critical 3.4 Mb Region on 17q24 Reveals Association Between Multiple Sclerosis and the PRKCA Gene. *S.P. Kallio*¹, *J. Saarela*¹, *R. Asselta*^{1,2}, *D.C. Chen*³, *A. Jokiahho*³, *A. Palotie*^{3,4,5,6}, *L. Peltonen*^{1,7}. 1) Dept of Molec Med, National Public Health Inst, Helsinki, Finland; 2) Dept of Biol and Genet for Medic Sci, Univ of Milan, Italy; 3) Dept of Hum Genet, School of Med, UCLA, CA, USA; 4) Finnish Genome Center, Helsinki, Finland; 5) Dept of Clin Chem, Univ of Helsinki, Finland; 6) Dept of Pathology, School of Med, UCLA, CA, USA; 7) Dept of Med Gen, Univ of Helsinki, Finland.

Multiple sclerosis (MS) is a chronic neurological disorder of the CNS. An increased prevalence (200/10⁵) of MS has been documented in the region of Southern Ostrobothnia in Western Finland. We have established linkage in 28 Finnish multiplex MS families to four loci, one on 17q22-q24. We further restricted the initial 23 cM locus to a 3.4 Mb region by combining power of linkage, LD, and monitoring for haplotype sharing among affected individuals. A set of 61 SNPs covering this region was genotyped in 22 large and 42 trio families originating from the high-risk MS region. Seven SNPs showing nominal evidence for association were further genotyped in an independent set of 439 Finnish trios. A SNP located in a large intron of the PRKCA gene showed the strongest evidence for association in these trios ($p=0.003$) and in a combined subset of families originating from the high-risk region ($p=0.00009$). The association with PRKCA was recently replicated in an English MS study sample. To capture most of the variation in this region and identify the MS predisposing alleles, we genotyped 250 additional SNPs in PRKCA and 15 other genes located in the critical region and monitored for differential expression in peripheral blood lymphocytes of 8 MS cases and 9 family controls. Interestingly, in addition to the PRKCA SNP, 4 SNPs located 0.5 Mb apart, in the HELZ gene also showed some evidence for association in the complete data set. These SNPs were observed to be in LD with the originally identified PRKCA SNP. Preliminary expression data indicated no differences for the steady state transcript level of regional genes between MS cases and controls, suggesting that the associated allele does not have a direct effect on transcription, at least not in the analysed cells.

Linkage and association analyses of adiponectin serum level in Type II diabetes. *IP. Kovac¹, R.J. Havlik², D. Foley², R. Peila², A. Singleton², J. Hardy², J. Egan³, D. Taub³, B. Rodriguez⁴, K. Masaki⁴, D. Curb⁴, FW. Fujimoto⁵, AF. Wilson¹.* 1) Dept Genometrics, NHGRI, Baltimore, MD; 2) NIA, Bethesda, MD; 3) NIA, Baltimore, MD; 4) University of Hawaii, Honolulu, HI; 5) University of Washington, Seattle, WA.

Type 2 diabetes is a common genetically influenced multifactorial disorder, affecting an estimated 8.6 million Americans over age 60 alone. The expression of adiponectin, an adipocyte-derived protein, correlates negatively with total fat [Arita et al. 1999], and lowered plasma levels of adiponectin were found in patients with Type 2 diabetes [Hotta et al. 2000]. Evidence of linkage for serum levels of adiponectin (LOD = 3.2) was reported at the chromosome 14, 29cM [Commuzzie et al. 2001]. Our objective was to evaluate linkage and association of adiponectin serum levels in this candidate region, using an independent sample in which marker D14S297 (31.75cM) showed some evidence of linkage and association with Type 2 diabetes. The data were derived from a sample of older Japanese Americans with a high prevalence of diabetes living in Hawaii, including 529 siblings from 175 families. Model-independent sib-pair linkage analyses were performed on microsatellite markers D14S297, D14S1040, and D14S1032, with SIBPAL [SAGE 4.5]; covariates included age, sex, and body mass index (BMI). Allelic association analyses for each marker were performed with ASSOC [SAGE 4.5]. Genotypic association analysis compared genotypic distributions at each marker between approximate lower and upper 10-th percentile, e.g. adiponectin serum level ≤ 4 (N = 53) versus ≥ 21 (N = 52), using Monte Carlo simulation of Fisher's exact test [SAS]. There was no evidence of linkage. Some association results were nominally significant, i.e. marginal Monte Carlo genotypic association at the marker D14S1032, 23.2cM ($p = 0.05$; $p = 0.04$ when upper and lower 20%-ile were compared for this marker only). No statistical evidence persisted after adjusting for multiple testing. The relatively small sample size may contribute to the ambiguous results concerning the hypothesis that regulation of adiponectin expression may contribute to Type 2 diabetes in our population.

Independent association of the MICA and DRB1 genes with Graves' disease (GD) in Taiwanese children. *YJ. Lee^{1,3}, CY. Huang¹, MR. Chen¹, FS. Lo², CW. Dang¹, FM. Chang¹, HF. Liu¹, CC. Chu¹, M. Lin¹.* 1) Dept Pediatrics & Medical Res, Mackay Memorial Hosp, Taipei; 2) Division of Endocrinology, Department of Medicine, Chang Gung Children's Hospital, Taoyuan; 3) College of Medicine, Taipei Medical University, Taipei; Taiwan.

MICA-TM A5 and DRB1*09012 are associated with GD. This study investigated which renders the stronger association. **Methods:** 158 children with GD and 333 healthy adults were genotyped for MICA-TM microsatellite and DRB1 gene. The stronger association was investigated according to Svejgaard. **Results:** MICA-TM A5 (Factor **A**) and DRB1*09012 (Factor **B**) in patients and controls (only significant comparisons are listed in the Table. OR: odds ratio, Pc: corrected p)

Conclusion: MICA-TM A5 & DRB1*09012 are independently associated with GD & DRB1*09012 shows a stronger association.

Comparison	a	b	c	d	OR	Pc
A vs non-A	116	42	178	155	2.41	0.00014
B vs non-B	82	76	88	245	3.00	1.79E-7
++ vs -+	70	12	60	28	2.72	0.0498
++ vs +-	70	46	60	118	2.99	4.18E-5
++ vs --	70	30	60	127	4.94	7.06E-9
in patient	70	46	12	30	3.80	0.0037
in control	60	118	28	127	2.31	0.0111

Genome Wide Allelic Association Study of Alcohol Consumption Behavior in a Finnish Population. *P.A. Lind¹, C.J.P. Eriksson², K.C. Wilhelmsen¹*. 1) Dept Genetics, University of North Carolina, Chapel Hill, NC; 2) Department of Mental Health and Alcohol Research, National Public Health Institute, Helsinki, Finland.

Analysis of population isolates with increased allelic and locus homogeneity may facilitate the identification of genes that predispose to alcoholism. We are attempting to map susceptibility genes relevant to alcoholism phenotypes by allelic association analysis in a population from Finland. In this study, male subjects (n = 305) were either recruited during clinical treatment for alcoholism (104 cases) or by advertisements (97 cases, 104 controls). All were screened with the Alcohol Use Disorders Identification Test (AUDIT) and information regarding alcohol-related aggression was recorded. Factor analysis of the AUDIT data identified two principal components that reflect alcohol consumption and social consequences of drinking. DNAs were assembled into 23 pools based on the AUDIT factor analysis, traditional assessment of the AUDIT score and patterns of aggression. DNA pools were screened for allelic association with 811 microsatellite markers with an average spacing of 4.6 cM, and an average heterozygosity of greater than 77%. Genotype data were corrected for differential amplification, stutter peak and pool-size affects; and pair-wise analyses of pool allelic frequencies were performed. Nominally significant allele image pattern and allelic differences between pools were detected with approximately 100 microsatellites. The population was individually genotyped for 34 markers that showed the greatest evidence of allelic association ($P < 0.001$) between alcoholism phenotypes and concordance with respect to both the associated allele and the direction of association between related pools. Pool allele frequencies calculated from the individual genotype data validated the pooled results for 25 markers (74%) and a high false positive rate was observed with less robust microsatellites. We found evidence for allelic association in six chromosomal regions and the association was reinvestigated by fine mapping with additional microsatellite markers and single nucleotide polymorphism typing of eight candidate genes in these regions. SLC6A3 and ALDH1A1 showed the greatest association.

Investigation of IL-1 System Genes in Risk for Childhood-Onset Mood Disorders. *V.L. Misener¹, P. Luca¹, K.G. Wigg¹, N. King², A. Vetro³, E. Kiss³, K. Kapornai³, J.L. Kennedy², M. Kovacs⁴, C.L. Barr^{1,5}*, *The International Consortium for Childhood-Onset Mood Disorders.* 1) Toronto Western Research Institute, Toronto, ON, Canada; 2) Neurogenetics Section, Centre for Addiction and Mental Health, Toronto, ON, Canada; 3) Department for Child and Adolescent Psychiatry, Szeged University, Szeged, Hungary; 4) University of Pittsburgh, Pittsburgh, PA, USA; 5) The Hospital for Sick Children, Toronto, ON, Canada.

Inflammatory cytokines, such as IL-1, IL-6 and TNF-, induce a syndrome known as "sickness behaviour", the symptoms of which overlap considerably with symptoms typically seen in depression. This has led to the hypothesis that cytokine activity is a contributing factor in depression. While there are correlative findings to support this, the identification of functional variants of cytokine system genes conferring risk for depression would clearly establish causality. To this end, we are testing such genes as candidates for involvement in childhood-onset mood disorders, making use of a large sample of families identified through probands affected by depression in childhood, a highly familial and heritable form of depression. A study of IL-1 system genes is underway. Transmission/disequilibrium test analyses show no evidence for biased transmission of alleles of the intronic 86-bp VNTR polymorphism of the IL-1Ra gene (4-repeat: 68 times transmitted *vs.* 70 times not, $p=0.865$; 2-repeat: 71 times transmitted *vs.* 64 times not, $p=0.547$). However, for the promoter region -511C/T polymorphism of the IL-1 gene, transmission of the T allele is more uneven (91 times transmitted *vs.* 72 times not), although the transmission bias does not reach statistical significance ($p=0.137$). Interestingly, this allele was recently found to be associated with depressive symptoms in both Alzheimer's disease and schizophrenia. Based on these findings, we are now investigating a functional variant at position -31 of the IL-1 gene that is reported to be in linkage disequilibrium with the -511T allele. Additional polymorphisms of the IL-1Ra and IL-1 genes, and other IL-1 system genes, including IL-1 and the IL-1 receptors, are also being tested for association with depression in our sample.

Association studies of DTNBP1, NRG-1, RGS4, MRDS1, G72/G30 and DAAO in an extended Irish schizophrenia

case - control sample. *D.W. Morris¹, K.A. McGhee¹, K. Murphy¹, S. Schwaiger¹, J.M. Nangle¹, G. Donohoe¹, S. Clarke¹, P. Baldwin², P. Scully², J. Quinn², D. Meagher², J.L. Waddington³, M. Gill¹, A.P. Corvin¹.* 1)

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We have previously published association analyses of dysbindin (DTNBP1; Morris et al. Schizophr Res 2003, Williams et al. Arch Gen Psychiatry 2004), neuregulin-1 (NRG-1; Corvin et al. Mol Psychiatry 2004) and regulator of G-protein signalling 4 (RGS4; Morris et al. Am J Med Genet 2004) in a schizophrenia case - control sample taken from the Irish population. The sample consisted of 249 cases and 231 controls. We have since extended this sample to consist of 299 cases and 645 controls. A sample of this size provides 80% power with 95% confidence to detect association with an odds ratio of 1.6. We have used this more powerful sample to test associations we previously reported at the DTNBP1, NRG-1 and RGS4 loci. In addition we have tested for evidence of association at the MRDS1 (OFCC1) locus at 6p24.3, at the G72/G30 locus at 13q34 and at the D-amino acid oxidase (DAAO) locus at 12q24. The results in brief are; DTNBP1 association with the P1655-P1635-SNPA haplotype (sim p = 0.006). NRG-1 no association with the HapBIRE haplotype or the core haplotype first reported in the Icelandic population. RGS4 association with RGS4-4 RGS4-7 RGS4-18 haplotype (p = 0.04). MRDS1 no evidence of association. G72/G30 - single marker associations with rs3916965 (p=0.005) and rs2391191 (p=0.01) in addition to haplotype associations with these markers. DAAO - association with rs2111902 (p=0.02) and rs3918346 (p=0.003) and haplotype associations with these markers.

The dopamine transporter gene (DAT1) core promoter polymorphism -67T variant is associated with schizophrenia. *M. Ohadi¹, F. Fadaei², N. Khodayari¹, M. Garshasbi¹, A. Rahimi³, L. Hafizi¹, A. Ebrahimi¹, H. Najmabadi¹.* 1) Genetics Research Center, Social Welfare and Rehabilitation Sciences University, Daneshjoo Blvd. Koodakyar St., Evin, Tehran 19834, Iran; 2) Razi Psychiatric Center, Shahr-e-Ray, Tehran 18664, Iran; 3) Sina Hospital Psychiatric Center, Hamedan Medical Sciences University, Hamedan, Iran.

Dysfunction of the central dopaminergic neurotransmission has been suggested to play an important role in the etiology of schizophrenia. The dopamine transporter (DAT1) mediates the active reuptake of dopamine from the synapses and thereby plays a key role in the regulation of the dopaminergic neurotransmission. In this study, we sought to determine the possible association of the DAT1 gene core promoter polymorphism -67A/T with schizophrenia in a case/control study. The allele and genotype frequencies of the polymorphism were studied in 100 patients and 100 controls, which were matched on the basis of sex, age and ethnicity. The genotype frequencies in the patients group were as follows: AA 29%; AT 59%; TT 12% vs. the genotype frequencies in the control group: AA 57%; AT 38%; TT 5% [$\chi^2 = 16.54$, $df = 2$, $OR = 2.25$ (95% CI 1.46-3.45, $p = 0.0003$)]. For the first time, these findings provide tentative evidence for the contribution of the DAT1 gene core promoter polymorphism to the etiopathophysiology of schizophrenia at least in the Iranian male population that we studied. Replication studies of independent samples and family-based association studies are necessary to further evaluate the significance of our findings.

Further investigation of a potential psoriasis susceptibility locus on chromosome 5q31. *L. Samuelsson¹, C. Friberg¹, S. Nilsson², J. Wahlstrom¹.* 1) Clinical Genetic, Univ Gothenburg, Sahlgrenska Univ Hosp/East, Gothenburg, Sweden; 2) Chalmers University of Technology, Gothenburg, Sweden.

Psoriasis is a chronic inflammatory disease of the skin with both genetic and environmental risk factors. In a genome-wide screen on a Swedish family set several candidate regions were identified. With the aim of decreasing the heterogeneity of the family set a stratification criteria based on joint involvement was applied. This led to increased non-parametric linkage values (P 0.01) for five chromosomal regions including one on chromosome 5. On chromosome 5, the microsatellite marker reaching highest NPL-value in the genome scan (P = 0.008) was D5S816. This marker is located at chromosome 5q31, a region where many genes involved in immune and inflammatory system are located. The same region has also been suggested to harbor susceptibility genes for several inflammatory or autoimmune diseases including Crohn disease, atopic dermatitis, rheumatoid arthritis and celiac disease. Of these, both Crohn and celiac disease have been reported to have an increased prevalence in individuals with psoriasis. There is also a possibility of a shared disease-gene between rheumatoid arthritis and psoriasis arthritis since the highest linkage value was reached in the cohort of families with psoriasis and joint involvement. All these potential susceptibility loci are located within 12 cM from D5S816. We have therefore started to investigate the chromosome 5q31 region more closely in a psoriasis cohort with the attempt to confirm our first finding. We have genotyped additional microsatellite- and SNP markers across the region and evaluated the result by family-based linkage- and association analysis.

Maximizing the use of founder effect in search for genetic predisposing loci for schizophrenia. *T. Varilo*^{1, 2}, *R. Arajarvi*^{1, 2}, *T. Hiekkalinna*¹, *T. Partonen*^{1, 2}, *J.D. Terwilliger*³, *J. Lonnqvist*^{1, 2}, *L. Peltonen*^{1, 2, 4}, *T. Paunio*^{1, 2}. 1) Depts. of Molecular Medicine, and Mental Health and Alcohol Research, National Public Health Institute, Haartmaninkatu 8, 00290 Helsinki, Finland; 2) Depts. of Medical Genetics and Psychiatry, University of Helsinki, Haartmaninkatu 2b, 00029 HUS, Helsinki, Finland; 3) Dept. of Psychiatry and Columbia Genome Center, Columbia University, 1150 St Nicholas Ave, New York 10032, USA; 4) Dept. of Human Genetics, David Geffen School of Medicine, University of California, Los Angeles, 90095-7088, USA.

Anticipated allelic and locus heterogeneity hampers attempts to identify predisposing loci for common, polygenic diseases. We have previously carried out three genome-wide scans in samples of Finns ascertained for schizophrenia from highly extensive national epidemiological registers. We have focused on an outlying small founder municipality on the eastern border of Finland with a well established 300-year-old population history. Genetic analysis of schizophrenia families from this isolate have provided evidence of putative predisposing loci on several chromosomes 1q, 2q, 4q, 9q and X, a result typical for complex diseases. In order to diminish genetic and environmental heterogeneity to its minimum, we proceeded by selecting families from municipality level from separate villages, and divided our families based on the genealogy and established population history. We observed some divergence between the numbers of the alleles in the microsatellite markers of the Southern and the Northern villages implying for genetic diversity between these populations. 26 families informative for schizophrenia originating from the Southern villages highlighted the highest genome-wide evidence for linkage on chromosome 4q ($Z_{\max} = 3.3$ versus < 1 in the families from the Northern villages), whereas 15 families from the Northern villages provided most of the linkage information on 9q ($Z_{\max} = 2.64$ versus 1.18 in the Southern villages). The linkage to 4q23 is of particular interest since we recently reported a susceptibility locus for verbal learning and memory to the same chromosomal region (Paunio et al. 2004).

Lack of association of the mitochondrial aspartate/glutamate carrier *SLC25A12* gene with autism or with markers of mitochondrial dysfunction in autistic patients. A.M. Vicente¹, C. Correia¹, A. Coutinho¹, L. Diogo², M. Grazina³, C. Oliveira³, G. Oliveira². 1) Instituto Gulbenkian de Ciência, Oeiras, Portugal; 2) Hospital Pediátrico de Coimbra, Coimbra, Portugal; 3) Centro de Neurociências, Coimbra.

In a population-based study of autistic children, we have previously found hyperlactacidemia in 20.3% of the tested patients, suggestive of mitochondrial dysfunction. Altered mitochondrial function will result in defects in the brain bioenergetic metabolism and in impaired CNS development and functioning, and therefore may be underlying autism. Mitochondrial disease was confirmed in 7.2% of the autistic patients with hyperlactacidemia and the absence of mtDNA mutations, indicates involvement of nuclear genes. A strong association of autism with two SNPs within the *SLC25A12* gene, a nuclear gene encoding a mitochondrial aspartate/glutamate carrier (AGC1), has been found. AGC1 plays an important role in the malate/aspartate shuttle, which regulates the cytosolic redox state controlling lactate to pyruvate conversion. We examined the association of two *SLC25A12* polymorphisms, rs2056202 and rs11757, with autism and markers of mitochondrial dysfunction, namely hyperlactacidemia and high lactate/pyruvate ratio. We found no transmission disequilibrium of either SNP alleles (rs2056202: $Z=0.873$; $P=0.383$; rs11757: $Z=0.816$; $P=0.414$) or haplotypes ($\chi^2=3.05$; 3df; $P=0.384$) in a sample of 241 nuclear families. No association of either SNP with lactate levels (rs11757: $P=0.404$; rs2056202: $P=0.5323$) or with lactate/pyruvate ratio distribution (rs11757: $P=0.2561$; rs2056202: $P=0.8784$) was found in 199 autistic subjects. Finally, no significant differences in allele or haplotype frequencies were found between autistic patients with confirmed mitochondrial disorder and healthy controls. In conclusion, we show that *SLC25A12* polymorphisms can not explain the high frequency of mitochondrial dysfunction markers in our sample and we do not replicate the recent findings of an association of rs2056202 with autism.

Identification of a common 3' UTR haplotype in AQP5, a candidate gene for human asthma. *S.L. Zimmerman, A.A. Staton, C.M. Krane.* Dept Biol, Univ Dayton, Dayton, OH.

Aquaporins (AQPs) are water channels that allow for the vectorial movement of osmotically driven water across cell membranes. AQP5 is a water selective channel expressed in the lacrimal, salivary, and airway submucosal glands, corneal and airway epithelium, and in type 1 pneumocytes. Genetically targeted AQP5 knockout mice are hyperresponsive to cholinergic-stimulated bronchoconstriction, a hallmark of asthma. Therefore, we hypothesized that a subset of individuals with clinical features of asthma and bronchoconstriction will have DNA mutations within the AQP5 gene. SSCP was used to screen DNA from 100 asthmatic patients, and 50 individuals from the population (25 Caucasian and 25 African American) for nucleotide variation in 368 ntds. of the 5' flanking region, 5' and 3' UTRs, and exons 1-4 of the hAQP5 gene. Six nucleotide variants were identified. A TA-GC transition identified in intron 1 and a GC-CG transversion in intron 2 are present in the asthmatic population with allele frequencies of 3.0% and 0.5% respectively. A GC-AT transition in exon 4 (2.0%) results in a synonymous substitution (Thr-Thr). Three SNPs were identified in the 3' UTR; an AT-TA inversion (2.0%) and two AT-GC transitions. The AT-GC transitions occur in linkage disequilibrium with a haplotype frequency of 9.5%/10.0%/6.0% in the asthmatic/ Caucasian/ African American populations. The final A to G change alters a conserved adenosine residue located two nucleotides downstream of the polyadenylation-cleavage site, which is considered to be critical in the formation of the polyadenylation complex. No individual was identified as a compound heterozygote or homozygote for the variants screened. An allelic discrimination method using real-time PCR was also developed to identify the 3' UTR polymorphisms in large-scale screens. **CONCLUSION:** A genetic mutation screen of the human AQP5 gene identified six nucleotide variants, including a common 3' UTR haplotype for which 18.0% of the population is heterozygous. The AT-GC transition variant located within the region of assembly of the polyadenylation complex may be functionally important in the post-transcriptional regulation of the AQP5 gene.

Replication and CpG Methylation Mediate FRAXA (CGG)_n Deletions in Primate Cells. *M.R. Blondin^{1,2}, K. Nichol Edamura¹, C.E. Pearson^{1,2}*. 1) Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Department of Molecular & Medical Genetics, University of Toronto, Toronto, Ontario, Canada.

Instability of the fragile X (FRAXA) CGG tract is known to be sensitive to both repeat length, purity and CpG methylation. Tracts 25 pure repeats can be unstably transmitted, but expanded and aberrantly methylated tracts (even heterogeneous patterns) are somatically stable. It has been suggested that for germline and somatic tissues, the absence of aberrant CpG methylation may enhance CGG deletions. For example, FRAXA males have only premutation CGG lengths in their sperm and rare high-functioning methylation mosaic FRAXA patients display high-levels of CGG length heterogeneity.

Using an SV40 primate DNA replication system, it was possible to investigate the effect of repeat length, direction and location of replication initiation, as well as CpG methylation upon CGG repeat stability in cells. Deletions were observed with 53 CGG repeats when replication was initiated proximal to the repeat tract using CGG strand as the lagging strand template. Replication mediated the presence of deletions, as no mutations were observed in the absence of replication. When replication was initiated further from the repeat while maintaining CGG as the lagging strand template or with templates using CCG as the lagging strand template, regardless of location of replication initiation, deletions were not observed. CpG methylation of the unstable template stabilized the repeat tract, decreasing the number and magnitude of deletion events. Templates with 20 CGG repeats were stable under all circumstances. These results reveal that CGG deletions occur during replication and are sensitive to replication fork dynamics, tract length, and CpG methylation.

Connections between FMRP and Rac1 pathways in mammalian cells. *M. Castets*¹, *A. Schenck*¹, *E. Bechara*¹, *H. Moine*², *E.W. Khandjian*³, *T. Rabilloud*⁴, *J.L. Mandel*¹, *B. Bardoni*¹. 1) Department of Molecular Pathology, IGBMC, ILLKIRCH, FRANCE; 2) IBMC, UPR 9002 CNRS, STRASBOURG, FRANCE; 3) Universite Laval, CHUQ, Quebec, CANADA G1L 3L5; 4) CEA, DRDC, LBCP, Grenoble, FRANCE.

Fragile-X syndrome, the most common form of inherited mental retardation, is caused by the absence of FMRP, a RNA-binding protein implicated in the regulation of mRNA translation and/or transport. A few hundreds of putative mRNA targets has been identified, but the specificity of interaction between FMRP and most of these mRNAs remains to be confirmed. Moreover, the consequences of the absence of FMRP upon expression of the corresponding proteins, as well as correlations with phenotypic features, are not fully determined. Using genetic analyses in *Drosophila*, we recently showed that dFMR1 is linked via dCYFIP, one of its interactor, to dRac1 pathway. Rac1 is a key regulator of actin cytoskeleton dynamics, with a well-established role in maturation and maintenance of actin-rich synaptic structures called dendritic spines. Strikingly, one of the main abnormalities observed in patients brain is an abnormal shape and number of these spines. To study Rac1-FMRP connection(s), we have established here a cellular model consisting of mouse fibroblasts cell lines stably expressing *FMR1* or not. Using this model, we demonstrate that FMRP-Rac1 link does exist in mammalian cells and we characterize parts of the molecular mechanisms connecting the two proteins. We show that activation of Rac1 by PDGF induces relocalization of FMRP and some of its partners close to actin structures called actin rings, which are involved in membrane protrusions. Moreover, Rac1-induced actin remodeling is enhanced in *FMR1* null fibroblasts. This phenotype is correlated with alterations in the level of proteins involved in Rac1 downstream signaling. FMRP binds with high affinity to the mRNA encoding one of these proteins, and is then very likely to negatively regulate its translation. Thus, we propose that the molecular mechanisms unraveled here, pointing to FMRP as a modulator of actin dynamics, could explain dendritic spine abnormalities and subsequently cognitive impairment in patients suffering from Fragile-X syndrome.

Association of Nucleotide Variations in The Apolipoprotein B48 Receptor Gene (APOB48R) with Plasma Total Cholesterol Levels in Hypercholesterolemic Individuals. *T. Nakajima*¹, *Y. Fujita*^{1, 2}, *Y. Ezura*¹, *A. Mi-i*^{1, 2}, *T. Bujo*³, *T. Kaneko*⁴, *K. Kamimura*⁴, *Y. Iino*², *Y. Katayama*², *S. Oikawa*², *Y. Saito*³, *M. Emi*¹. 1) Dept. Molecular Biology, Inst. Gerontology, Nippon Medical School, Kawasaki, Japan; 2) Dept. Internal Medicine, Nippon Medical School, Tokyo, Japan; 3) Departments of Genome Research and Clinical Application, and, of Clinical Cell Biology, Chiba University Graduate School, Chiba, Japan; 4) Awa Medical Association Hospital, Chiba, Japan.

Multiple factors predispose to the phenotypic features of high serum cholesterol levels, though details are not clearly defined yet. Here we investigated a possible association between the genotypes of variations in the apolipoprotein B48 receptor gene (APOB48R) and plasma cholesterol levels in 352 adult hypercholesterolemic patients in Japan. Six variations in APOB48R including c.1036-1062/del27 and A419P were analyzed for the phenotypic association to the age and gender-adjusted levels of total cholesterol (T-Cho), LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C). Analysis of variance (ANOVA) with linear regression analysis detected a significant correlation between the genotypes of the A419P variation and the adjusted T-Cho levels ($r = 0.15$, $p = 0.007$). T-Cho levels among three genotypically categorized individuals, i.e., 265 homozygous A-allele carriers (243.4 \pm 21.4 mg/dl), 78 heterozygous carriers (247.9 \pm 23.7 mg/dl) and 9 homozygous P-allele carriers (263.1 \pm 21.1 mg/dl) indicate a co-dominant T-Cho highering effect of the minor C-allele. Similar effect was detected for c.1036-1062/del ($r = 0.13$, $p = 0.015$). Linkage disequilibrium (LD) analyzed on 8 variations including the neighboring loci indicated a significant LD within the locus. These results indicate that genetic variations in APOB48R are the important factors affecting lipoprotein phenotypes of the hypercholesterolemia. Our data providing a new insight for etiological studies of the hypercholesterolemic diseases may suggest a novel aspect of the mechanism that has to be considered in its pathogenesis and modifications in clinical manifestations.

***Drosophila* VDAC: A model system to study mitochondrial function.** *B.H. Graham, E. Alesii, W.J. Craigen.*
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Voltage-dependent anion channels (VDACs) are a family of small pore-forming proteins of the mitochondrial outer membrane found in all eukaryotes. VDACs play an important role in the regulated flux of metabolites between the cytosolic and mitochondrial compartments and three distinct mammalian isoforms have been identified. The specific physiologic and potential pathophysiologic roles of the various isoforms are not understood, but animal and cell culture experiments suggest that the various isoforms function in apoptosis, learning and reproduction. Annotation of the *Drosophila melanogaster* genome has revealed three putative VDAC isoforms (*CG17137*, *CG31722-PA*, *CG31722-PB*) closely linked to the previously reported *Drosophila* VDAC (*porin*). Molecular characterizations reveal complex developmental and adult sex-specific expression patterns for these genes. When expressed in yeast deficient for YVDAC, *porin* and *CG17137* rescue a conditional-lethal phenotype. Electrophysiological characterization of these proteins in reconstituted systems demonstrates a differential ability to permeabilize lipid bilayers and liposomes. Analysis of flies homozygous for hypomorphic P element alleles of *porin* or with knockdown of *porin* expression *in vivo* by RNAi reveal abnormal phenotypes including partial lethality and altered viability, neuromuscular dysfunction and male infertility-phenotypes reminiscent of mouse VDAC mutants. Transgenic expression of *porin* in the homozygous, hypomorphic background rescues the partial lethality and neuromuscular phenotypes. These studies demonstrate that *Drosophila* VDAC homologs have retained important aspects of VDAC structure and function, warranting further studies to utilize this genetic model system in delineating fundamental conserved roles of VDACs and mitochondria in eukaryotic cell function.

Investigation of polymorphisms in genes involved in mitochondrial function and oxidative phosphorylation for association to AD. *R.T. Perry¹, H. Wiener¹, L.E. Harrell¹, D. Blacker², R.E. Tanzi², M. McInnis³, S.S. Bassett³, R.C.P. Go¹.* 1) Univ Alabama at Birmingham, Birmingham, AL; 2) Massachusetts General Hospital, Charlestown, MA; 3) Johns Hopkins University, Baltimore, MD.

The brain is noted for its high oxygen consumption and the mitochondria is the location for cellular oxidative phosphorylation (OXPHOS). Normal mitochondrial function is important in neuronal development and structure, including affecting axonal and synaptic activity, however, mitochondrial respiration is the chief source of ROS in the cell. There is increased oxidative damage to nucleic acids and mitochondria in AD brains due to free radicals and ROS. Mitochondrial dysfunction is a hallmark feature of A-induced neuronal toxicity in AD. Recent studies using microarray technology to examine the differential expression of thousands of genes in the brains of mice overexpressing APP compared to normal mice, have identified a set of genes that are involved in mitochondrial energy metabolism and programmed cell death pathways. We recently reported the significant associations of two single nucleotide polymorphisms (SNPs) in the SOD2 gene, a key enzyme involved in the detoxification of the superoxide radical, to AD patients in the NIMH AD Genetics Initiative cohort and to an African-American case-control data set. Interestingly, the SOD2 gene is located at 6q27, a chromosomal candidate region of interest identified in AD genomic scans. Because of the above evidence, we have genotyped polymorphisms in additional genes shown to be important for normal mitochondrial function and nuclear and mitochondrial OXPHOS genes. Some of the nuclear genes reside in chromosomal candidate gene regions detected in the genomic scan we reported on last year. We will present the results of the genotypings of the SNPs in these genes at the meeting and discuss what role they may play in AD pathogenesis.

Genetic screen for mitochondrial phenotypes in mouse embryonic stem cells. *C. Stromberger, B.H. Graham, W.J. Craigen.* Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

Mitochondrial encephalomyopathies are important causes of mental retardation and multisystem disease in humans. Recent epidemiological evidence suggests that the minimal prevalence of mitochondrial diseases is 1 in 8500. Despite important insights into the clinical, biochemical, and molecular characterization of these disorders, specific genetic etiologies have been identified in only a minority of cases, and the underlying molecular pathogenesis remains poorly understood, with virtually no effective therapies available. We have devised a strategy for performing a genetic screen in mouse embryonic stem (ES) cells designed to identify genes that are important for mitochondrial function and that are potential etiological candidates for mitochondrial diseases. ES cells mutagenized by a retroviral-mediated promoter-trap strategy are screened by fluorescence-activated cell sorting (FACS) for abnormal mitochondrial phenotypes using fluorescent markers for mitochondrial mass and mitochondrial membrane potential. A small scale, pilot mutagenesis experiment has been performed and has resulted in the isolation of a promoter-trap clone that demonstrates a 31% decrease in the mean fluorescence for the mitochondrial mass marker and a 53% increase in mean fluorescence for the mitochondrial membrane potential marker. This result demonstrates that this approach can result in the isolation of mutant ES clones with potential aberrant mitochondrial phenotypes as suggested by fluorescent markers. By combining the genetic tractability of mouse ES cells with the high-throughput screening capacity of FACS, this proposal offers a novel, rapid approach for identifying genes important for mitochondrial function in a mammalian system well suited for studying mitochondrial biology. The identification of abnormal mitochondrial phenotypes in mutagenized mouse ES cells offers the promise of identifying candidate genes for human disease, generating new animal models for studying the pathophysiology of mitochondrial disease and delineating potential therapeutic targets.

Genetic evidence for a mitochondrial etiology of Alzheimer Disease (AD). *D. Wallace, E. Ruiz-Pesini, P. Coskun.* MAMMAG, Univ of California, Irvine, Irvine, CA.

The genetic etiology of late-onset AD remains unknown, though multiple studies have reported defects in mitochondrial oxidative phosphorylation (OXPHOS). We have discovered a germline mitochondrial DNA (mtDNA) mutation in the tRNA^{Gln} gene at np 4336 that is present in 5-7% AD patients but 0.4% controls, and that ancient cold-adaptive mtDNA missense mutations which uncouple mitochondrial OXPHOS and increase heat production are protective of AD, presumably because they reduce mitochondrial reactive oxygen species (ROS) production. Mitochondrial ROS induces somatic mtDNA mutations, and we have found a 15 fold increase in mtDNA rearrangements in AD brains. Moreover, we have discovered that the mtDNA control region (CR) mutation T414G, which affects the light (L)-strand promoter, is present in 65% of AD brains but 0% controls and that all mtDNA CR mutations are increased 63% in AD brains {79% in 59-69 year old (YO) patient brains, 18% in 70-79 YOs, and 130% in over 80 YOs}. Moreover, certain mtDNA mutations, such as the AD-specific T477C, can increase to 70-80% of the AD brain mtDNAs in the 70-79 YOs. Finally, the mtDNA L-strand transcript for ND6 and the mtDNA copy number are reduced 50% in AD brains. These observations indicate that AD is the result of the accumulation of somatic mtDNA mutations which result in OXPHOS deficiency, synaptic loss and dementia; a conclusion supported by recent reports that ApoE 4 and A increase mitochondrial ROS production.

Phenotypic, genetic and developmental characterization of recessive ENU-induced mouse models of cleft palate.

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We observe a large spectrum of recessive phenotypes similar to human congenital disorders, including several craniofacial defects, in a late embryonic ENU mutagenesis screen. Clefting occurs in many known mouse mutants, but those with isolated clefts are ideal models of human non-syndromic cleft lip/palate (NSCL/P) (1/500-1000 live births). We have identified the *cleft palate only 1* (*cpo1*; isolated cleft secondary palate) and *curly tail/cleft palate* (*ctcp*; cleft secondary palate and/or a curly tail) mutations that are excellent such models.

The *cpo1* mutant phenotype is caused by a mutation that affects splicing efficiency of a zinc finger transcription factor on chr 4 (Hs. 1p36). A specific defect in palatal shelf elevation is evident upon histologic evaluation of *cpo1* mutants. *cpo1* gene expression during craniofacial development is consistent with its playing an important role in palatogenesis. 1p36, near *CPO1*, has been implicated in the etiology of human non-syndromic clefting. We identified three potential etiologic missense mutations in a screen of 200 NSCL/P cases from Iowa and the Philippines that are not observed in 200 controls. A *CPO1* haplotype is in strong linkage disequilibrium with Filipino non-syndromic cleft lip only and shows a weaker association with Iowa non-syndromic cleft palate only, thereby supporting an important role for the *CPO1* gene in the complex etiology of NSCL/P.

We identified a causative nonsense mutation in a novel protocadherin gene on chr 3 (Hs. 4q28) in *ctcp* mutants. *ctcp* gene expression during development is consistent with the mutant phenotype. Identification of downstream target genes and interacting proteins for these genes is in progress. We are also utilizing lentiviral-expressed RNAi to rapidly knock down these genes in mouse embryos and provide confirmation of the etiology of these ENU-induced mutations.

Mouse knockout analysis confirms and elucidates regulators of cell proliferation. *C. Friddle, R. Finch, A. Abuin.*
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Deletion of Ku80 (aka Ku86) results in the decreased proliferative capacity of the affected cells (Vogel et al. 1999), illustrating the role of Ku80 as a positive regulator of cell proliferation. Loss of p53 allows fibroblasts to continue replicating past the point at which wildtype cells normally cease to divide (Reddell 1998), showing that p53 is a negative regulator of cell proliferation.

Despite the clear importance of these two proteins as regulators of cell proliferation, their mode of biological function, via protein-protein interactions, has proven very difficult to modulate by traditional pharmaceutical compounds (Chene 2004). We seek to circumvent this difficulty by identifying downstream members of the cell proliferation pathway that meet more traditional criteria for pharmaceutical intervention (i.e. 'druggable', Zambrowicz and Sands 2003).

Using the Affymetrix U74v2 chips, we screened for candidate genes that were up- or down-regulated in Mouse Embryonic Fibroblasts deficient in p53 or Ku80. Fibroblasts were cultured from p53 and Ku80 $+/+$ and $-/-$ embryos as previously described (Lim et al. 2000) and RNA was extracted. Comparison of RNA expression levels in two knockout samples versus four wildtype samples yielded dozens of genes whose expression levels were markedly changed. However, substantial changes in gene expression do not demonstrate a causative role in the pathway or disease.

Candidate genes that possess druggable protein domains in addition to substantial changes in gene expression were queued for mouse knockout analysis as part of our Genome5000 program. Knockouts were generated using either homologous recombination or our OmniBank library of gene-trapped mouse embryonic stem cells (www.mouseknockout.com). The phenotypic data presented here demonstrates that at least some of the candidate genes we identified as potential regulators of cell proliferation play a causative role and that the specific function of a gene in this pathway cannot be predicted by the direction and magnitude of the changes in gene expression. Mouse knockouts prove to be a powerful tool for both confirming the role of a gene in a disease process and determining its normal function.

A psoriasis-associated, synonymous SNP of the corneodesmosin (CDSN) gene affects transcript stability by altering the affinity for an RNA binding-protein. *F. Capon¹, P. Hobby², M. Allen³, A.D. Burden⁴, D. Tillman⁴, J.N. Barker³, R.C. Trembath¹.* 1) Dept of Genetics, University of Leicester, Leicester, United Kingdom; 2) The Randall Institute, King's College, London, United Kingdom; 3) St Johns Institute of Dermatology, Kings College, London, United Kindom; 4) Department of Dermatology, Western Infirmary, Glasgow, United Kingdom.

Psoriasis is a common skin disorder presenting with a multifactorial aetiology. Genome scans have provided compelling evidence for a major disease susceptibility locus on chromosome 6p21 (PSORS1) and refinement studies have defined a critical interval containing three highly polymorphic genes (HLA-C, HCR and CDSN) that carry psoriasis-associated alleles. We have previously obtained genetic evidence supporting a role for CDSN variation in psoriasis susceptibility. On this basis, we have now undertaken an assessment of CDSN SNP biological impact. We observed that the SNPs that are exclusive to psoriasis-associated CDSN haplotypes are located in gene regions implicated in the stabilization of RNA transcripts. CDSN being over-expressed in psoriatic lesions, we hypothesised that disease-associated intragenic SNPs may affect the rate of mRNA decay. Using transfection-based assays, we were able to demonstrate that the stability of mRNAs transcribed from CDSN risk haplotypes was increased by twofold. Site-directed mutagenesis showed that a single synonymous SNP (CDSN*971T) accounts for the augmented RNA stability. CDSN*971T localises to a RNA stability motif and UV cross-linking analysis demonstrated that it affects the affinity for a 39kDa RNA binding protein. Modelling of the corneodesmosin three-dimensional structure indicated that amino-acids modified by substitutions in cis of CDSN*971T are exposed on the protein surface, where they might alter corneodesmosin antigenicity. Finally, association analyses showed that haplotypes bearing CDSN*971T confer psoriasis susceptibility in a wide range of ethnic groups.

Hyperprolinemia is a risk factor for schizoaffective disorder. H. Jacquet¹, C. Demily^{1, 2}, E. Houy^{1, 2}, B. Hecketsweiler³, J. Bou¹, G. Raux¹, J. Lerond⁴, G. Allio², S. Haouzir², A. Tillaux⁵, C. Bellegou², G. Fouldrin², P. Delamillieure⁶, S. Dollfus⁶, T. D'Amato⁴, M. Petit^{1, 2}, F. Thibaut^{1, 2}, T. Frebourg¹, D. Champion^{1, 2}. 1) Inserm U614, Faculty of Medicine, Rouen, France; 2) Department of Psychiatry, Rouvray Psychiatric hospital and Rouen University Hospital, 76031 Rouen, France; 3) Department of Biochemistry, Rouen University Hospital, 76031 Rouen, France; 4) EA 3092, University Claude Bernard, IFR Neurosciences Lyon, 69677 Bron, France; 5) Dieppe Hospital, 76200 Dieppe, France; 6) Centre Esquirol, Caen University Hospital, 14000 Caen, France.

DNA sequence variations within the 22q11 DiGeorge chromosomal region (DGCR) are likely to confer susceptibility to psychotic disorders. In a previous report we identified several heterozygous alterations, including a complete deletion, of the proline dehydrogenase (*PRODH*) gene which were associated with moderate hyperprolinemia in a subset of DSM III schizophrenic patients. We have now conducted a case-control study including 114 control subjects, 188 patients with schizophrenia, 63 with schizoaffective disorder and 69 with bipolar disorder to determine whether hyperprolinemia is associated with increased susceptibility for any of these psychiatric conditions. We report that, taking into account a confounding effect due to valproate treatment, hyperprolinemia is a risk factor for DSM III schizoaffective disorder ($p= 0.02$, OR= 4.6, 95% CI 1.3-16.3). We did not detect 22q11 interstitial deletions associated with DiGeorge syndrome among the 320 patients included in this sample and we found no association between common *PRODH* polymorphisms and any of the psychotic disorder. In contrast, we found that five rare *PRODH* alterations (including a complete *PRODH* deletion and four missense substitutions) were associated with hyperprolinemia. In several cases, two variations were present simultaneously, either in *cis* or in *trans* in the same subject. Eleven from 30 hyperprolinemic subjects bore at least one genetic variation associated with hyperprolinemia. This study demonstrates that moderate hyperprolinemia is an intermediate phenotype associated with certain forms of psychosis.

Novel NOD2 Deletion in an Inflammatory Bowel(IBD) Patient. *R. Colliton¹, D. Melvin¹, E. Fan¹, R. Baldassano², B. Kamath², K. Loomes², D. Piccoli², C. Stolle².* 1) Pathology and Lab Medicine, Children's Hospital of Phil, Philadelphia, PA; 2) G.I. and Nutrition, Children's Hospital of Phil, Philadelphia, PA.

Crohn disease (CD) and ulcerative colitis(UC) are chronic inflammatory bowel diseases(IBDs)that are usually easily differentiated from one another based on clinical findings. In approximately 10% of cases, classification remains difficult. The NOD2 gene encodes a protein involved in bacterial recognition by monocytes and has been identified as one of the Crohn disease susceptibility loci. Three SNPs in the NOD2 gene,(R702W, G908R and 1007fs) have been associated with CD but not UC. We have screened a population of IBD patients for these SNPs using PCR and DNA sequencing analysis. In the course of screening, DNA from one patient with severe IBD failed to amplify with primers specific for the 3' end of exon 4 (ie. the R702W SNP), while exons 8 and 11 amplified. To determine whether a polymorphism under one of the primers was responsible for the PCR failure, primers for the 3' end of exon 4 were redesigned. The redesigned primer sets also failed to amplify suggesting a homozygous intragenic deletion of NOD2. Since primers for exons 5 and for the 5' end of exon 4 amplified, the breakpoints of the deletion must be limited to the 3' end of exon 4 and the 3' end of intron 4, a region of approximately 4.2 kb. To further delineate the extent of the deletion we designed a series of primers along IVS4. We have thus far been unable to amplify across the presumed breakpoint, indicating that most or all of this region is deleted. The exact breakpoints of this deletion are currently being determined but are likely to include the 3' end of exon 4 and at least 700bp of intron 4. A mutation of this nature has not yet been reported and we hypothesize that its effects would be similar to the 1007fs protein truncating mutation seen in CD patients, which is associated with a severe phenotype in homozygotes. The frequency of this mutation in the population is not known since heterozygotes would not be detected with the present SNP assay. However, inheritance of this mutation alone or together with another NOD2 variant may help explain the severity of the CD phenotype in some patients with IBD.

Lysyl oxidase genes in Cutis laxa. V. Hucthagowder^{1,3}, P. Coucke², K. Fong¹, K. Csiszar¹, Z. Urban^{1,3}, *The Cutis Laxa Consortium*. 1) Laboratory of Matrix Pathobiology, John A Burns School of Medicine, University of Hawaii, Honolulu, HI 96822, USA; 2) Department of Medical Genetics, Ghent University hospital, De Pintelaan, B-9000 Ghent, Belgium; 3) Department of Pediatrics, Washington University School of Medicine, St. Louis, MO 63110, USA.

Cutis laxa (CL) is a heterogeneous group of inherited or acquired disorders which shows distinct inheritance patterns (autosomal dominant, recessive and X-linked) suggesting that CL may be caused by mutations in multiple loci. Deficiency of lysyl oxidase activity has been reported to be associated with CL. The goal of this study was to investigate whether molecular defects in lysyl oxidase genes may cause CL. We collected 40 families with CL and applied the denaturing high performance liquid chromatography (DHPLC) methodology to screen lysyl oxidase (LOX) and lysyl oxidase-like (LOXL) genes for detecting mutations. The observed variants were also sequenced using ABI 3100 for further confirmation. We identified 21 variants in our CL probands. One variant in the exon 1 of the LOX gene was due to a G/A transition and resulted in a nonconservative arginine to glutamine substitution (R158Q). The genotype frequencies for R158Q were significantly different between CL patients (n=40) and controls (n=121), (chi square test, p 0.025). There were more R/Q heterozygotes and Q/Q homozygotes among the CL patients. In the LOXL gene 4 missense variants (R141L, G153D, A159S, A160P) and two silent variants (875CT and 960GT) were observed in exon 1. 14 other variants were located in different intronic regions and 3- untranslated region of the LOXL gene. Current studies include mutational analysis of the proline rich region of the LOXL, analysis of allele frequencies in normal individuals and functional studies on the expression and enzyme activity of lysyl oxidases in CL patients.

Searching for mutations in the *EFHC1* gene in patients with juvenile myoclonic epilepsy. P.A.O. Ribeiro¹, V.D.B. Pascoal¹, L.E. Betting², N.F. Santos¹, R. Secolin¹, S.B. Mory², C. Guerreiro², M. Guerreiro², F. Cendes², I. Lopes-Cendes¹. 1) Dept Medical Genetics, UNICAMP, Campinas, SP, Brazil; 2) Dept Neurology, UNICAMP, Campinas, SP, Brazil.

The idiopathic generalized epilepsies (IGE), for which a genetic cause is widely accepted, account for 39 to 59% of all epilepsies. The most common form of IGE is juvenile myoclonic epilepsy (JME). The first epilepsy locus was mapped in JME on chromosome (ch) 6p and ever since, in spite of almost two decades of study, the results are still conflicting. The *EFHC1* gene, mapped on ch 6p12-p11, could be responsible for the JME phenotype, since this gene contains an EF-hand motif, suggesting a role in neuronal calcium equilibrium. It spans approximately 63 kb, contains 11 exons and encodes 640 aminoacids. Recently, putative mutations were described in exons 2, 3, 4 and 5 of this gene in a few families segregating JME.

We investigated the relevance of the *EFHC1* gene in the etiology of JME in a large sample of patients.

All patients were diagnosed according to the ILAE classification of epilepsies and epileptic syndromes. We performed mutation analysis of the *EFHC1* gene in 20 patients with JME and 20 controls. All individuals were genotyped by PCR using five pairs of primers that amplified the entire coding region and exon/intron boundaries of exons 3, 4 and 5 of the *EFHC1* gene. PCR products were subsequently analyzed by the single-stranded conformation polymorphism (SSCP) method.

SSCP analyses did not detect any differences in pattern of migration between patients and controls.

We have not identified any mutations or sequence variants in the three exons of the *EFHC1* gene genotyped in our group of JME patients. We are still completing the mutation screening of the entire coding region of the *EFHC1* gene in order to better determine its potential role in the etiology of JME in our group of patients.

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Evaluation of the FGF signaling pathway in nonsyndromic cleft lip and palate. *B.M. Riley, B. Trump, E. Russo, S. Santiago, A.R. Vieira, J.C. Murray.* Dept Pediatrics, Univ of Iowa.

Nonsyndromic cleft lip and palate (NS CLP) is a complex multifactorial birth defect resulting from a combination of genetic and environmental factors. In the Philippines, NS CLP has a birth prevalence of 1 in 500 and large families provide the opportunity for gene mapping and localization. A genome-wide linkage scan including 220 NS CLP Filipino families identified a novel region on chromosome 8p21, which includes the fibroblast growth factor receptor 1 (FGFR1) gene. Subsequent sequencing of candidate genes in the region in 90 Iowa NS CLP and 180 Filipino NS CLP patients identified three potential disease-causing mutations in FGFR1. Mutations in FGFR1 are known to cause craniosynostosis and the autosomal form of Kallman syndrome (anosmia/infertility) in which approximately 5% of patients have clefts. A nonsense mutation, R609X, was identified in a two-generation family from Iowa originally diagnosed with isolated CLP. Further contact with this family revealed that the proband developed features of Kallman syndrome but that her father, who has the R609X genotype, has isolated cleft lip and palate as a phenotype. Additionally, two missense mutations, M369I and E467K, have each been found in two NS CLP Filipino families and in 0 out of 1400 controls. Other identified mutations include 3 synonymous, 2 common non-synonymous, and 20 non-coding variants from introns and UTR. Conserved mouse-human homology regions lying 5' and 3' of FGFR1 were sequenced and 1 variant in 1000 bp of sequence was identified. Micro-deletion assays were also performed, however, there is no evidence of a deletion in the 200 cases screened. The interest in FGFR1 and its role in clefting extended the project to other interacting members of the family, including FGFR2, FGFR3, FGF2, FGF4, FGF8, FGF9, and FGF10. Two novel missense mutations have been identified in FGFR2 in Filipino families. We have also begun sequencing FGF10 and several variants have been found in the Filipino population. Further investigation of the FGF/FGFR family in NS CLP patients may reveal a role for FGF signaling in the development of cleft lip and palate and allow studies of gene-gene interaction.

Sequence analysis of MSX1 gene in brazilians patients with nonsyndromic cleft lip with or without cleft palate.
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Cleft lip and/or palate (CL/P) are a major congenital structural anomaly that is notable for significant lifelong and complex etiology. Approximately 2/3 of cases does not show any others anomalies and are called non-syndromic (NS). The incidence of NS-CL/P is approximately 1 in 700 live born and the preliminary studies have suggested a handful of genes and/or loci in humans, as well as environmental disrupters appear to play a role in the genesis of such cases. The frequent occurrence as well as their extensive psychological, surgical, speech and dental involvement emphasize the importance of understanding the underlying causes. The MSX1 gene has been proposed as a gene in which mutations may contribute to NS-CL/P. Support to this comes from human linkage and linkage disequilibrium studies. Some previous sequencing studies revealed some mutations that can play a role in the etiology of the NS-CL/P. In this study we sequenced the MSX1 gene in 60 patients with NS-CL/P. The results revealed 2 missense mutations (75 G-) in a conserved block and (101 C-) and 1 polymorphism (48C-) on exon 1. This may show that mutations can be find identified in some subjects with NS-CL/P although it remain for future studies with parents and relatives to confirm if these particular mutations are causal.

Mutational studies on PRODH gene located in VCFS critical region of 22q11 in patients with schizophrenia. Y.

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The gene proline dehydrogenase (PRODH) encoding for 59 kDa protein is located in the low copy repeat regions of chromosome 22q11. PRODH is involved in the first step of metabolic conversion from proline to glutamate, making it a relevant protein in neural functioning. This along with the location of this gene in the VCFS region makes it a logical candidate for schizophrenia. In an earlier study Jacquet et al (AJHG 11: 2243-49) identified two rare mutations L441P (exon 12) and L289M (exon 8) in patients with schizophrenia that were not seen in the control samples. We have used their exon 12 primers (5'-TGACAATGTGACCCTGGACG-3' and 5'-CATGACATAAAAGCTGAGGAAATA-3') to assess any sequence variation in a group of schizophrenia patients from southwestern Ontario, Canada and their age, sex, ethnic group matched controls. All patients and controls were recruited and assessed by a single psychiatrist (R.O'Reilly) using DSM IV. We have identified a total of 8 missense mutations. Some of these mutations represent known SNPs and most of the amino acid substitutions involve phylogenetically conserved sites. 10 of 19 patients and 4 of 17 matched controls were found to have at least one base substitution. We report novel mutations at the E428G, A430V and R432H. One patient had 9 base changes, 7 of these involved amino acid substitutions, considered amplified of the pseudogene. This observation in repeated experiments from this patient only (and no other individual) however remains to be explained. We conclude that genetic variation in the PRODH gene is common. Based on their distribution, some of the substitutions (R431H) represent polymorphism, while others (A472T, A430V) remain logical causal mutations, but remains to be established. Supported by Schizophrenia Society of Ontario and Ontario Mental Health Foundation.

Polyglutamine-mediated changes in the biochemical function of TBP. *X.J. Li, M. Friedman, S.H. Li.* Human Genetics, Emory University, Atlanta, GA.

Transcriptional dysregulation has been proposed as a molecular mechanism for the pathogenesis of the polyglutamine (polyQ) diseases. Study of spinocerebellar ataxia 17 (SCA17), the most recently identified polyQ disease, may provide insight into this mechanism. The mutant protein in SCA17 is the TATA-binding protein (TBP), which mediates transcription by all three eukaryotic RNA polymerases as a fundamental component of several different complexes. To investigate the effect of the N-terminal polyglutamine tract on TBP function, we have generated TBP cDNA constructs containing 15, 31, 71, and 105 CAGs. When expressed in HEK 293 cells, only mutant TBP (71 and 105 CAGs) formed nuclear aggregates. Preliminary data from reporter assays suggest that the transcriptional impact of a polyglutamine expansion in TBP may be dependent on promoter strength and type. Specifically, for TATA-containing promoters, the effect of the polyQ expansion was inversely related to the strength of the promoter. We found that polyQ expansion alters the interaction of TBP with TATA-box DNA. Relative to the wild type protein, 71Q TBP bound less promoter DNA despite a reduced tendency for dimerization. However, expansion of the polyQ stretch increased the interaction of TBP with the transcription factor Sp1. These results indicate that polyQ expansion may affect multiple functions of TBP, thereby facilitating transcriptional dysregulation and ultimately contributing to SCA17 pathogenesis. Currently, we are trying to generate a transgenic mouse model of SCA17 in order to confirm these polyQ-mediated changes in TBP function in vivo.

POLYMORPHISMS IN FKBP5, A CO-CHAPERONE OF THE GLUCOCORTICOID RECEPTOR ARE ASSOCIATED WITH RESPONSE TO ANTIDEPRESSANT DRUGS. *M.A. Kohli¹, D. Salyakina¹, G. Wochnik¹, S. Lucae¹, M. Ising¹, B. Puetz¹, N. Kern¹, P. Lichtner², T. Bettecken², J.C. Mueller², E. Lohmussaar², T. Meitinger², T. Baghai³, B. Bondy³, R. Rupprecht³, M. Uhr¹, T. Rein¹, F. Holsboer¹, B. Mueller-Myhsok¹, E.B. Binder¹.* 1) Max-Planck Institute of Psychiatry, Munich, Germany; 2) Institute for Human Genetics, Technische Universitaet, Munich and GSF, Neuherberg, Germany; 3) Department of Psychiatry, Ludwig-Maximilians-University Munich, Germany.

It is hypothesized that antidepressants might all target a common final signaling pathway. Due to consistent dysregulations of stress hormones in the brain and peripheral circulation of depressed patients, the hypothalamic-pituitary-adrenal (HPA) system is considered a strong candidate. We found a strong association of SNPs in FKBP5, a glucocorticoid receptor (GR)-regulating co-chaperone of hsp90 with response to antidepressant drugs. By fine-mapping about 300kb around this gene, we could show that only SNPs located within the major LD-block containing FKBP5 were highly associated with response to antidepressant treatment ($p=0.00003$). We genotyped the three SNPs with the strongest association in our sample ($N = 280$) in a replication sample ($N = 85$). Two of the three SNPs showed a significant association with response to antidepressant treatment in the replication sample. In both samples, the association of response reflects an overrepresentation of homozygotes of the minor allele of the associated SNPs among the responders. Homozygosity for the minor alleles was also correlated with a higher protein expression of FKBP5 in lymphocytes and with a lower ACTH response in a neuroendocrine test assessing HPA-axis activity. The association of FKBP5 polymorphisms with response to antidepressant drugs appears to be independent of the primary pharmacological profile of antidepressants, as similar associations were obtained when dividing patients into three groups treated with different classes of antidepressants. These results support that FKBP5 and by extension the HPA-system may be involved in the common final signaling pathway of antidepressant drugs.

A major influence of CYP2C19 genotype on the metabolism of N-desmethylclobazam, an anticonvulsant of benzodiazepine class. *K. Kosaki¹, K. Maeyama³, K. Tamura¹, R. Sato², H. Samejima¹, Y. Tanigawara², T. Takahashi¹.*
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Introduction N-desmethylclobazam (NCLB), the major metabolite of the anti-epileptic drug clobazam (CLB), exerts a large influence on the therapeutic and adverse effects of CLB. A substantial inter-individual variability has been observed in the ratios of NCLB concentration/CLB dose and of the NCLB/CLB concentration. In the present study, we tested whether CYP2C19 genotype was associated with an altered metabolism of CLB and NCLB among 22 patients receiving CLB, giving careful consideration to a unique Japanese population structure in which the *2 and *3 alleles are the prevalent (35% altogether) and the exclusive mutant alleles. **Methods** A group of 22 patients (1.5 to 33 yrs, median=6.5 yrs) who had received oral administrations of tablets or granules of CLB with the dose unchanged at least for 4 wks at the time of study were studied after obtaining written informed consent. Genomic DNA was isolated from whole blood, and CYP2C19*2 and CYP2C19*3 mutations were detected by direct sequencing. **Results** The NCLB/CLB concentration ratio in 5 patients with two non-functional alleles (3 homozygotes for *2 or 2 compound heterozygotes for *2 and *3) were six-fold higher ($p=0.0001$) than those of 7 patients with normal alleles (*1 homozygotes). Patients with one non-functional allele (10 heterozygotes for *2 or *3) exhibited intermediate trait. Similarly, NCLB concentration/CLB dose ratio elevated as the allele count of the non-functional allele increased ($p<0.0001$). **Conclusion** We document here a genotype-phenotype correlation between CYP2C19 polymorphisms and those ratios. Patients with two mutated CYP2C19 alleles show significantly higher ratios than those with the wild type genotype: patients with one mutated allele exhibited intermediate an intermediate trait. That is, the degree of elevation in the ratios was dependent on the number of mutated alleles of CYP2C19 (gene-dose effect).

Association and Interaction Effects of Six Serotonin Pathway Genes to Fluoxetine Response in Major Depression.

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Several lines of evidence suggest that indirect effects on the neurotransmission of serotonin are associated with the therapeutic action of SSRIs. Thus, it is plausible that genetic variants in serotonin receptors (i.e., HTR1A, HTR2A, and HTR2C) and enzymes responsible for the biosynthesis (TPH1 and TPH2) and degradation (MAOA) of serotonin may influence a patient's response to fluoxetine. We have previously reported an association between publicly available SNPs in a number of these candidate genes and antidepressant response phenotypes in our well-characterized clinical sample consisting of 96 subjects with major depression treated with fluoxetine. In order to capture further genetic diversity or functionally relevant variants in serotonin pathway genes in these samples, we resequenced the coding regions, intron-exon boundaries, and 5' proximal conserved non-coding regions of these 6 candidate genes in this well phenotyped clinical sample. In total, we analyzed 30kb per sample and identified 115 polymorphisms, 71 of which have not been previously described. The majority of SNPs uncovered via resequencing were rare, with 49 of them detected on only a single chromosome in our population. Genetic diversity and LD patterns were variable among the 6 candidate genes. Single locus association analysis was significant between global clinical antidepressant response and two SNPs (in TPH1 [p=0.02] and MAOA [p=0.02]). Our previously published haplotype associations seen with the HTR2A, TPH2, and TPH1 genes remained significant (p=0.03-0.0001) after addition of haplotype tagging SNPs uncovered during resequencing. In addition to single gene effects, we will present analysis of interaction/combined effects of polymorphisms in the serotonin pathway on fluoxetine response, using Classification and Regression Tree (CART) and multivariate logistic regression.

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"Medication Response in Obsessive-Compulsive Disorder and the Genetics of Hepatic Enzyme CYP2C19 Metabolism". *M. Richter, T. Sicard, P. Arnold, E. Burroughs, J. Kennedy*. Centre for Addiction and Mental Health, University of Toronto, Toronto, Ontario.

There is considerable evidence supporting a genetic basis for Obsessive-Compulsive Disorder (OCD). To date, most work has focused on candidate gene hypotheses based on etiological models of the illness. An alternative strategy, little used to date, is to investigate the genetics of drug response. In this study, retrospective data regarding response to selective serotonin reuptake inhibitor (SSRI) medication trials was collected in a sample of 20 individuals meeting DSM-IV criteria for OCD. All individuals were genotyped for alleles 2 and 3 in the hepatic enzyme 2C19. In total, 12 of the 17 individuals with the common wild type genotype for alleles 2 and 3 were responders. One out of 3 individuals with a genotype characterized by at least one rare variant for allele 2 responded to medication. A rare mutant allele that has been identified for the 3 allele did not appear in our sample. There were no statistically significant between-group differences demonstrated in this small preliminary investigation (Fisher's exact test, $p=.270$). However, given the potential clinical benefits of identifying genotypes that predict response to medication, further work is merited.

Haplotypes of angiotensin II receptor genes are associated with blood pressure (BP) reduction in response to ACE inhibitor therapy in Chinese Hypertensives. X. Su¹, L. Lee², X. Li¹, J. Lu², Y. Hu², S. Zhan², W. Cao², L. Mei¹, Y. Tang¹, D. Wang¹, JI. Rotter¹, R. Krauss³, H. Yang¹. 1) Dept Medical Genetics, Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) Peking Univ, Health Sci. Ctr; 3) Children's Hosp. Oakland Res. Inst., Oakland, CA.

Angiotensin-converting enzyme inhibitors (ACEI) are among the drugs as first-line therapy for hypertension. However, there is marked inter-individual variation in BP response to ACEI. To identify genes that may contribute to this variability, we tested the relation of ACEI BP response to two candidates, the angiotensin II receptors AGTR1 and AGTR2, in a Chinese sample. The sample included 901 hypertensives who participated in a Chinese community based trial using ACEI benazepril for 3 yrs. We genotyped 6 SNPs from AGTR1 and 3 from AGTR2. Multivariate analyses were performed for single SNPs and haplotypes (HAPs), using BP reduction as the outcome variable, and age, gender, dose, and other medication as covariates. Of 26 AGTR1 HAPs, 3 were common: H1 59.9%/H2 20.7%/H3 17.1%. AGTR1-H2 was associated with increased BP reduction while H3 was associated with decreased BP reduction. Means of SBP/DBP reduction (mmHg) for AGTR1 H2 and H3 carriers were 16.0/9.4 (n=143), 3.6/4.1 (n=64), respectively, and for the remainder, 10.9/7.5 (n=680). In analysis with covariates, AGTR1 HAPs were independently associated with BP reduction (p<0.0002 for both SBP & DBP). Of 6 AGTR2 HAPs, 3 were common: H1 59.9%/H2 20.7%/H3 17.1%. In women, we observed an association between AGTR2 and BP reduction. Means of SBP/DBP reduction for AGTR2 H2 and H3 carriers were 9.6/4.9 (n=103) and 19.5/9.3 (n=81), respectively, and for the remainder, 12.8/6.0 (n=140) (SBP p=0.001; DBP p=0.004). In a model including all covariates, sex*AGTR2 interaction, and two genes, both AGTR1 and AGTR2 were independently associated with SBP (p<0.0003) and DBP (p<0.03). BP reduction response to ACEI is independently associated with both the AGTR1 and AGTR2 genes. These associations are being tested in a confirmatory sample from the same population with the goal of confirming that AGTR1 and AGTR2 haplotypes can be used to predict clinical response to ACE inhibitor therapy.

A novel cysteinyl leukotriene 1 receptor (*CysLT₁*) variant is associated with atopy and asthma in a Tristan da

Cunha isolate. *M.D. Thompson*^{1,2}, *N. Zamel*³, *A. Slutsky*^{3,4}, *C. Lilly*⁵, *W.M. Burnham*¹, *D.E.C. Cole*^{2,3}, *K.A.*

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Asthma is a complex trait with genetic and environmental components, while atopy is a well-defined phenotype of immune hyperactivity that often underlies asthma. The *CysLT₁* receptor gene has been implicated in atopy, because it is: 1) the target for the cysteinyl leukotriene ligands known to activate eosinophils and mast cells; and 2) the target for *CysLT₁* receptor antagonists (e.g., montelukast) used to treat asthma. Located at Xq13.2-21.1, *CysLT₁* variants could also contribute to gender-based differences in atopic asthma in some populations. Accordingly, we analyzed the *CysLT₁* receptor gene to identify candidate single nucleotide polymorphisms (SNPs) that might contribute to asthmatic or atopic phenotypes, and found a novel c.899GA SNP predicting a G300S amino acid substitution. Genotyping was then conducted to determine allele frequency in the Tristan da Cunha isolate with an excess prevalence of atopy (47% of 112 subjects). Overall, the frequency of the 300S variant in this Tristan da Cunha sample was 15% (25/167), but the odds-ratio (OR, unadjusted for kinship) for atopy in 300S carriers was more than 6-fold higher (OR=6.28, 95%CI:2.2-17.7, p.001) than for those with only wild-type G300 alleles. For asthma itself, the increase in risk was not as dramatic, but stratification by gender suggested that most of the risk was attributable to the heterozygous females (16/57 carriers: OR=4.0, 95%CI:1.2-13.3). This association was unchanged when General Linear Modeling was used to correct for gender and cigarette smoking covariates. Whether this finding can be generalized to other populations remains to be determined.

Molecular characterization of 22q11.2 microdeletions by real-time quantitative PCR: new insights. R. Weksberg¹, J.A. Squire², L. Moldovan¹, E. Chow³, A.S. Bassett³. 1) Div of Clin and Metabolic Gen, Hosp for Sick Children, Toronto, ON, Canada; 2) Ontario Cancer Institute and Depts of Lab Medicine, Pathology and Medical Biophysics, Dept Molecular & Medical Genetics, Univ Toronto, Toronto; 3) Clinical Genetics Research Prog, Centre for Addiction and Mental Health, and Dept of Psychiatry, Univ Toronto, Toronto.

Chromosome 22q11.2 deletion syndrome (22q11DS) encompasses a heterogeneous group of clinical disorders including congenital malformations and schizophrenia. Most individuals with 22q11DS carry either a ~3Mb or a ~1.5Mb deletion diagnosed by a standard FISH assay using the TUPLE1 (Vysis) probe. However, some cases of 22q11DS carry unique deletions not detected by the routine diagnostic assay.

We have developed a real-time quantitative polymerase chain reaction (qPCR) assay for 22q11.2, firstly to detect unique deletions/duplications and secondly to better characterize 22q11.2 deletion endpoints. Since the 22q11.2 genomic architecture is characterized by dense polymorphic segmental duplication, we used a combined bioinformatics (*in silico*) approach together with both qPCR and FISH. A physical map was generated to identify 36 single copy primer sets across 5Mb of 22q11.2 for real-time qPCR analysis.

We tested DNA from 4 controls, 1 patient trisomic for 22q11 and 72 patients with clinical features of 22q11DS. Our real-time qPCR assay robustly detected both deletions and duplications of 22q11.2. Of 46 interstitial deletions that were identified by qPCR, 2 were not previously detected by FISH analysis using the TUPLE1 probe. The position of these 2 unique deletions was confirmed by FISH analysis using the RP11-138C22 probe. The breakpoints of these unique deletions were localized to regions containing extensive blocks of homologous segmental duplications; a subset mapped to regions with large numbers of Alu repeats. This observation expands the range of repeat sequences involved in the destabilizing event that generates 22q11.2 microdeletions.

These data demonstrate that real-time qPCR can increase the sensitivity of detection for 22q11.2 deletions and duplications and can be utilized to study the molecular etiology of such genomic alterations.

FREQUENCY OF HINDIII (LPL) POLYMORPHISM IN PATIENTS WITH CORONARY ARTERY DISEASE IN MEXICAN POPULATION. *L. Arnaud Jr¹, M.P. Gallegos-Arreola¹, L.E. Figuera², G.M. Zúñiga-González¹.* 1) Medicina Molec, CIBO IMSS, Guadalajara, Mexico; 2) Genética, CIBO, IMSS, Guadalajara, Jalisco, México.

INTRODUCTION. The coronary artery disease (CAD) is one the most important cardiovascular disorders and the principal world cause of death. For a lot of time have been revaluated different risk factors (genetics and environmental). Between genetics factors we can find modifications in lipoprotein lipase levels leading to elevated triglycerides and reduced high density lipoprotein (HDL), both of then are risk factors for coronary artery disease. Some studies have described an association between HindIII polymorphism and Lipoprotein Lipase (LPL) and CAD, although this association is contradictory in others studies and its association is unknown in Mexican population. **METHODS.** In this study, genomic DNA from 153 CAD patients (abnormal coronariography), 106 controls with (normal coronariography) and 136 persons without CAD and background of CAD, diabetes mellitus and arterial hypertension were genotyped. **RESULTS.** HindIII -/- polymorphism shown statistical differences compared with others population. Lipidic profile components were higher in the HindIII -/- group with regard to +/- and -/-. When groups were classified by gender, HindIII +/+ was associated with masculine gender in the cases group. **CONCLUSIONS.** HindIII -/- genotype could play an important role as risk factor of CAD in the group studied.

No Association or Linkage of a common polymorphism in the Cyclooxygenase 2 gene with Alzheimer disease.

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The potential contribution of cyclooxygenase (COX) in Alzheimers disease (AD) may be inferred from a number of studies. COX isoforms are expressed in the brain and are involved in physiological functions such as synaptic plasticity, prostaglandin production, and excitotoxicity. Individuals with no baseline dementia have a 60 to 80 % reduction in risk of developing AD when using non-steroidal anti-inflammatory drugs for at least two years. In addition, transgenic mice over-expressing COX-2 in neurons have accelerated deposition of amyloid in brain. Collectively, this evidence suggests that COX activity may be involved in the pathogenesis of AD. A recent study demonstrated that a common SNP polymorphism in the 3 untranslated region of COX-2 affects expression of COX-2. To test the hypothesis that COX-2 might be involved in AD, we genotyped 469 individuals affected with AD and 948 unaffected individuals from 249 families for this common polymorphism. We used the Pedigree Disequilibrium Test (PDT) to test for association between this locus and AD. The analysis of this SNP (hcv7550203 (rs5275)) did not show any significant linkage or association to AD. Although this polymorphism does not show an association with AD, we cannot exclude the possibility that other polymorphisms within COX2 might be related to AD. Other polymorphisms are currently being tested.

Investigating the role of prolactin and oxytocin system genes in childhood onset mood disorder. *J.M. Brathwaite¹, N. King¹, S. Shaikh¹, D. Voineskos¹, A. Vetr², E. Kiss², I. Baji³, C.L. Barr⁴, M. Kovacs⁵, J.L. Kennedy¹.*

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Childhood onset mood disorder (COMD) is a serious affective illness that alters the normal development of a child. We examined the role of oxytocin and prolactin system genes in COMD probands. These hormones play a major role in the quality of parent-child bonding across species and are therefore relevant to regulation of emotion in childhood. Oxytocin has been shown to be involved in stress coping strategies, while prolactin has been seen to reduce stress response. We examined polymorphisms of the oxytocin (OXT), oxytocin receptor (OXTR), prolactin (PRL) and prolactin receptor (PRLR) genes. Our samples were 1) 197 Hungarian families with an affected proband, and both parents, and 2) 200 cases collected in Pittsburgh, and matched controls. Diagnosis in these subjects was determined using the Interview for Children and Adolescents - Diagnostic Version (ISCA-D). To test for biased allele transmission from parent to COMD patient in our families, we employed the sibling-Transmission Disequilibrium Test (s-TDT). We had significant results for PRLR(T/C) ($\chi^2=4.921$, $P=0.027$) and OXTR(T/G) ($\chi^2=4.900$, $P=0.027$). For the OXT(T/G) there was a tendency towards biased transmission ($\chi^2=3.76$, $P=0.053$). Associations were observed in the case-control study for markers OXT(G/A) ($\chi^2=7.099$, $P=0.003$), OXTR(T/G) ($\chi^2=8.645$, $P=0.013$) and PRL(C/A) ($\chi^2=6.925$, $P=0.031$). Combining Hungary and Pittsburgh samples Z-scores for OXT and OXTR indicated association with OXT ($Z=3.25$, $P=0.00058$) and OXTR ($Z=3.64$, $P=0.0001$). Results for the other markers were not significant. These results suggest a role for the involvement of prolactin and oxytocin in the pathogenesis of COMD, and there is a need for further studies.

Polymorphisms of Apolipoprotein E: Association with Susceptibility and Severity of Multiple Sclerosis. V.

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The apolipoprotein E (APOE) polymorphism is known to impact on various neurologic disorders and has differential effects on the immune system and on CNS repair. Previous findings concerning a possible modulation of the clinical course of MS have been inconsistent, however. In a cross-sectional study, the authors investigated 81 patients with clinically definite MS and related their clinical and demographic findings to the allelic polymorphism of the APOE gene. The genotype distribution of patients with MS was compared with a cohort of 93 asymptomatic elderly volunteers. The authors found significant differences in the distribution of 4 allele between patients with MS and controls (9.3% vs. 0.5%; $\chi^2=15.2$; $df=2$; $p<0.001$). An analysis of disease progression in 81 patients with MS indicated that APOE-4 carriers are more likely to be affected with severe disease (61.5% with EDSS 7 vs. 7.7% and 30.8% with EDSS 3-7 and 3, respectively) ($\chi^2=7.88$; $df=2$; $p=0.019$). These results suggest effect of the APOE genotype on susceptibility to MS, and indicate an association of the APOE 4 allele with a more severe course of the disease.

ROR2 mutations in cleft lip and palate with limb defects. *M. Mansilla, S. Daack-Hirsch, J. Murray.* Dept Pediatrics, Univ Iowa, Iowa City, IA.

As part of a large cleft-candidate gene study conducted with subjects from the Philippines we have clinical data and biological samples from 49 individuals with orofacial clefting and some type of limb defect. Twenty-three of these subjects have amputation-type defects ranging from shortened terminal phalanges and nails to partial congenital limb amputation. Of the remaining 26 subjects, 7 have polydactyly, 5 mild syndactyly, 4 simian creases, 3 ectrodactyly, 3 clinodactyly, 2 club foot and 1 each joint contractures or broad thumbs. Many digital amputation-like defects appear similar to those described in the brachydactyly type B (BDB1) phenotype. In humans, mutations in ROR2 account for autosomal recessive Robinow syndrome and autosomal dominant BDB1 both include abnormalities of the limbs or digits. We detected 10 new variants. Eight are likely normal SNPs: two in the untranslated region A3734G and C3399T; four synonymous G1630A, C1885T, C2287T and C475T and two in the intronic region G223412A and G192488A. Two are missense mutations that are likely etiologic for the phenotype: E307L (exon 6) and G558S (exon 9). E307L was found in an affected proband, his affected cousin and his unaffected parents. This variant located at the extracellular domain, is likely to interfere ligand-receptor binding. G558S was found in a proband but not in her unaffected mother. This variant is located at the tyrosine kinase domain. Missense mutations in this region may inactivate the domain. We also examined a collection of nonsyndromic cleft lip with or without cleft palate (CL/P) cases. A 10-cM genome scan of 388 extended multiplex families with CL/P from seven diverse populations (2,551 genotyped individuals) revealed CL/P genes in six chromosomal regions, including a novel region at 9q21 (heterogeneity LOD score=6.6) near the ROR2 gene. To determine if ROR2 might contribute to non syndromic forms of CL/P we also sequenced exon 6 and exon 9 in 90 non-syndromic cleft samples and in 90 healthy controls, but did not find any etiologic variants. ROR2 mutations and genetic counseling should be considered in children with clefts and limb defects. ROR2 does not appear to be a common cause of isolated clefts.

ENDOTHELIAL NITRIC OXIDE SYNTHASE (eNOS) GENE POLYMORPHISMS IN CORONARY ARTERY DISEASE (CAD) PATIENTS OF MEXICAN POPULATION. *R. Mariaud-Schmidt¹, M.P. Gallegos-Arreola¹, L. Arnaud-López¹, L.E. Figuera², R. Arechavaleta³, T.J. Beltrán³, G.M. Zúñiga-González¹.* 1) Dept Medicina Molecular, CIBO, IMSS, Guadalajara, Mexico; 2) Dept Genética, CIBO, IMSS, Guadalajara, Jalisco, México; 3) Departamento de Endocrinología, HE, CMNO, IMSS, Guadalajara, Jalisco, México.

BACKGROUND: The vascular endothelium is now recognized as an important participant in a healthy cardiovascular system, and dysfunction of this cellular monolayer might be an initiating event in many or most cardiovascular disease states. eNOS gene polymorphisms have been associated inconsistently with cardiovascular disease. **OBJECTIVE:** Determination frequencies of three clinically relevant eNOS polymorphisms: T786C in the promoter, the variable number of tandem repeats (VNTR) in intron 4, and the Glu298Asp variant in exon 7 in Mexican population. **MATERIAL AND METHODS:** We study 205 CAD patients, subdivided in subgroup 1: 114 patients with coronary stenosis and subgroup 2: 91 patients without coronary stenosis and, a control group: 60 healthy individuals of the northwest Mexican population. For the polymorphisms analysis we amplified a 420bp segment of intron 4, a 180bp segment of promoter for T786C polymorphism, and a 248bp segment of exon 7 for Glu298Asp polymorphism. **RESULTS:** The genotype frequency of intron 4 (VNTR) were: 4a/4a, 27% in subgroup 1, 22% in subgroup 2, and 8% in the control group. The genotype frequency of T786C polymorphism were: C/C 16% in subgroup 1, 15% in subgroup 2 and 7% in the control group. The genotype frequency of Glu298Asp polymorphism were: 7% of T/T in subgroup 1, 4% in the subgroup 2 and 28% in the control group. **CONCLUSION:** This results suggest that the 4a/4a and C/C genotypes may be an important risk factors in CAD development in mexican population, and may be an useful in the effort to understand the polymorphisms associated with CAD and, its interpopulational diversity.

Two SNPs in the 5' and 3' end of the OPN (Osteopontin) gene contribute to susceptibility to Systemic Lupus

Erythematosus (SLE). *P. Momigliano-Richiardi¹, S. D'Alfonso¹, N. Barizzone¹, M. Giordano¹, A. Chiocchetti¹, C. Magnani¹, L. Castelli¹, F. Giacomelli², R. Scorza³, M.G. Danieli⁴, S. Migliaresi⁵, M.G. Sabbadini⁶, M. Galeazzi⁷, G.D. Sebastiani⁸, U. Dianzani¹.* 1) Univ. of Eastern Piedmont, Novara, Italy; 2) G. Gaslini Institute, Genova, Italy; 3) Milano Univ. , Milano, Italy; 4) Univ. Politecnica delle Marche, Ancona, Italy; 5) Napoli Second Univ., Napoli, Italy; 6) Vita e Salute Univ., Milano, Italy; 7) Siena Univ., Siena, Italy; 8) S. Camillo-Forlanini Hospital, Roma, Italy.

SLE is a multifactorial autoimmune disease. Several lines of evidence suggest that the early T-cell activation gene-1 (ETA-1) or osteopontin (OPN) has a role in the pathogenesis of SLE through its immunoregulatory effects. We tested the association of OPN with SLE by typing 394 Italian SLE patients and 479 matched controls for polymorphisms detected by scanning the coding, 5' and 3' flanking regions of the OPN gene by DHPLC in 23 SLE patients. Among the 13 detected SNPs, alleles -156G (0.714 vs 0.651, $p=0.006$, $p_c=0.036$) and +1239C (0.377 vs 0.297, $p=0.00094$, $p_c=0.0056$) were significantly increased in the patients. The presence of the associated allele in single or double dose conferred an OR=2.19 (95%CI 1.34-3.60) for SNP-156 and OR=1.53 (95%CI 1.14-2.05) for SNP +1239. A multivariate analysis showed that these effects were independent of each other. The risk associated to a double dose of susceptibility alleles at both SNPs was 3.8 (95%CI 2.0-7.4) fold higher relative to the complete absence of susceptibility alleles. A parallel and significant increase in OPN serum level (determined by ELISA) with increasing number of SLE susceptibility alleles was observed in 80 healthy individuals ($p=0.0019$). Considering individual clinical and immunological features, a significant association was seen for lymphadenopathy with -156 genotypes (overall $p=0.0011$; $p_c=0.046$). These data suggest the separate effect of a promoter (-156) and a 3'UTR (+1239) SNP in both SLE susceptibility and OPN serum production. We can speculate that these sequence variants (or others in perfect linkage disequilibrium) predispose to a high OPN production and that this in turn may confer susceptibility to SLE.

Investigation of a candidate gene for Inflammatory Bowel Disease on 12q23. *N.J. O'Callaghan¹, E.J. Walker¹, K.E. Peters¹, P.G. Board¹, J.A. Cavanaugh².* 1) John Curtin School of Medical Research, ANU, Canberra, AUSTRALIA; 2) MGRU, ANU Medical School, Canberra, AUSTRALIA.

Inflammatory bowel disease (IBD), encompassing Crohns disease (CD) and ulcerative colitis, is a group of debilitating diseases that involve chronic inflammation of the gut. Although the precise disease etiology of IBD remains unclear, numerous studies suggest a strong genetic component involved in disease susceptibility. One of the genomic regions identified as potentially harbouring IBD genes is the *IBD2* region on chromosome 12q. Here we report on suggestive linkage (NPL=2.1) at 12q23 in the Australian IBD population. This linkage is enhanced (NPL=3.0) in families carrying *CARD15* risk alleles. Seven polymorphisms within the region of maximal linkage were genotyped in 130 Australian IBD families. Single TDT (Transmission Disequilibrium Test) revealed a significant association with one polymorphism ($p < 0.05$) that is strongest in families carrying *CARD15* risk alleles ($p < 0.001$). The polymorphism is within an intron of a known gene involved in immune response. Quantitative Real-Time PCR analysis shows a disturbed pattern of expression; with an increase of mRNA levels detected in CD affected biopsy samples. We report the identification of a positional and functional disease candidate for IBD on chromosome 12. Our findings also suggest that this candidate interacts genetically with *CARD15*.

Association Analysis of 5HT Transporter Gene in Bipolar Disorder in the Colombian Population. *J.C. Prieto^{1,2}, C. Duran¹, A. Ordonez¹, J.F. Ortiz³, D. Patermina³, R. Tamayo³, F. Rivadeneira¹, C. Gomez².* 1) Inst de Genetica Humana, Univ Javeriana, Bogota, Colombia; 2) Hospital la Victoria; 3) Hospital San Ignacio.

Affective disorders are a common heterogeneous group of disease characterized by disturbances of mood. The bipolar affective disorder (BPD) is major psychiatric disorder that affects 1% of the population worldwide and it is characterized by episodes of mania and depression. Two polymorphisms in the serotonin transporter gene (5HTTR) have been examined in association studies of affective disorders. A polymorphic region linked to the gene 5HTTR has been associated with anxiety-related personality characteristics. A 17-bp repetitive element present in the second intron of the same gene also has been identified that consists of 9, 10, or 12 copies of the repeat sequence. In a Scottish study were found an association between the 9-repeat intronic VNTR allele, whilst in two other studies in European or British Caucasians the risk was associated with the 12-repeat allele. The objective of this study is to investigate association of the polymorphisms VNTR in intron 2 of 5HTTR gen. We have performed a case-control analysis in 75 cases with bipolar affective disorder and 81 control subjects. Allele frequencies for 12 and 10-repeat allele were different in the control subjects allele₁₂=0.43, allele₁₀=0.56 and cases allele₁₂=0.57, allele₁₀=0.42. Allele frequencies for 9-repeat allele were same in the control subjects allele₉=0.01 and cases allele₉=0.01. A significant association of the 12-repeat allele of the 5HTTR gene with bipolar disorder in Colombian population ($\chi^2=5.36$ $p<0.05$). Those results is similar an others studies in the European or British Caucasians. The 12-repeat allele may be a genetic risk factor in Colombian population.

Polymorphism interactions may influence asbestos-related disease development. *E.A. Putnam, A. Groves, K. Guttmanova, M.A. Pershouse.* Biomedical and Pharmaceutical Sciences/CEHS, University of Montana, Missoula, MT.

That polymorphisms contribute to phenotypic variation, disease risk, and response to the environment has long been hypothesized. A promising approach to dissect these contributions is the systematic exploration of the common gene variants that may be associated with disease. Although asbestos-related diseases (ARD) are among the most well studied occupational diseases, relatively little is known about the polymorphisms that may influence individual susceptibility. The goal is to investigate candidate genes, including those involved in inflammation and subsequent fibrosis, for polymorphisms that may be implicated in ARD development. DNA was isolated from 177 blood samples donated by Libby MT residents. Genotypes of GSTM1, GSTP1 and GSTT1, as well as TNF α (-308), IL1A, IL1B and IL1RA were determined. A general linear model of analysis was used to determine significance and possible interactions. A statistically significant main effect of IL1RA on disease scores was found ($F(2, 119)=3.127, p=.047$). Although no significant interactions were found, the interaction of GSTP1 and IL1RA accounted for 7% of the variance. The following four covariates were included in the model for their ability to predict disease: age, gender, exposure (0 to 3) and smoking as measured in pack/years. Gender had a statistically significant main effect ($F(1, 119)=4.823, p=.030$), as did smoking ($F(1,119)=8.050, p=.005$) and there was a significant interaction between age and exposure in the expected direction ($F(1, 119)=22.205, p<.001$). Overall the analyses have accounted for 40.4% of the variance; IL1RA by itself accounts for 5% of the variance. These data suggest that although the covariates play a large role in the development of asbestos-related diseases, they do not account for all of the variance. Results from the polymorphisms examined in the candidate genes thus far demonstrate the genetic contribution to ARD development, and analysis of the polymorphism interactions is promising. These studies demonstrate the need for a larger sample size to have enough power to detect significant contributions. Supported by ES011676.

Examination of several candidate genes on chromosome 10q in Alzheimer disease. *N.C. Schnetz-Boutaud¹, X. Liang¹, B.M. Anderson¹, J. Bartlett¹, E. Martin², W.K. Scott², M.A. Pericak-Vance², J.L. Haines¹*. 1) Center for Human Genetics Research, Vanderbilt University, Nashville, Tennessee; 2) Center for Human Genetics, Duke University Medical Center, Durham, North Carolina.

Alzheimer's disease (AD) is a genetically complex disorder that is the leading cause of dementia in the US. Although four genes have been identified that influence both early and late onset AD, these genes explain only part of the underlying genetic etiology. Numerous linkage studies have identified a large region on chromosome 10q that appears to segregate with AD. Despite follow-up efforts, the interval remains large and further localization has been difficult. Within this ~35 Mb region, numerous functional candidate genes have a strong biological rationale for examination in AD, and we chose five for additional analysis (Cdc2, Alpha Catenin, PLAU, TNFRSF6 and IDE). We used a dataset assembled from the NIMH repository, the NCRAD repository, Vanderbilt University, and Duke University. The total dataset consists of 4531 individuals (2491 affected, 1386 unaffected, 654 unknown) in 1184 families, 1152 cases and 320 unrelated controls. Multiple SNPs in each gene were selected for either their functional significance and/or their former use in other studies on AD. The combined analysis of the datasets identified a linkage signal in alpha-catenin with a lod=1.18 under a dominant model. The maximal lod score was 2.20, under the dominant model, in the NIMH subset. Association analyses using the Pedigree Disequilibrium Test did not demonstrate any significant results that survived even a modest correction for multiple comparisons. Additional SNPs in each gene, along with conditional analyses to account for the effects of APOE and family history, are ongoing. These data suggest that common polymorphisms in these candidate genes are not strongly associated with AD.

Genetic polymorphisms predict acute renal injury after coronary bypass graft surgery. *M. Stafford Smith, M. Podgoreanu, M. Swaminathan, B. Phillips-Bute, J.P. Mathew, R. Morris, M.F. Newman, D.A. Schwinn.* Dept Anesthesiology, Duke Univ Medical Ctr, Durham, NC.

Background: Post-cardiac surgery renal dysfunction is a common, serious multifactorial disorder, with inter-patient variability poorly explained by clinical, procedural, and biological markers. Therefore, we tested the hypothesis that selected gene variants are associated with acute renal injury after cardiac surgery.

Methods: 1671 patients undergoing aortocoronary surgery were studied. Clinical co-variables were recorded and DNA isolated from preoperative blood; MALDI-TOF mass spectrometry was used for genotype analysis. A model was developed relating clinical and genetic predictors to postoperative acute renal injury.

Results: A race effect was found so Caucasians and African Americans were analyzed separately. Overall, clinical factors alone are poor predictors of postoperative renal injury, although better in African Americans ($R^2=0.13$) than Caucasians ($R^2=0.03$). When 12 candidate polymorphisms are assessed, 2 demonstrate strong association with renal injury (>50% decrease renal filtration); these alleles include interleukin6 -572C and angiotensinogen 842C in Caucasians ($p<0.0001$ when they present together). Using less stringent criteria for significance ($0.01>p>0.001$), 4 additional polymorphisms are identified (apolipoproteinE 586T (2), angiotensin receptor1 1166C, and eNOS 894T in Caucasians; eNOS 894T and ACE D/I in African Americans). Adding genetic to clinical predictors resulted in the best model, with overall ability to predict renal injury increasing 4-fold in Caucasians and doubling in African Americans ($p<0.0005$).

Conclusions: In this study we identify, for the first time, genetic polymorphisms that collectively provide a 2-4-fold improvement over clinical factors alone in predicting post-cardiac surgery renal dysfunction. From a mechanistic perspective, most identified genetic variants are associated with increased renal inflammatory and/or vasoconstrictor responses.

Association of Proopiomelanocortin Gene Polymorphisms with Obesity in the IRAS Family Study. *B. Sutton*^{1,2}, *C. Langefeld*⁴, *A. Williams*⁴, *J. Norris*⁵, *M. Saad*⁶, *S. Haffner*⁷, *D. Bowden*^{1,2,3}. 1) Ctr Human Genomics, Wake Forest Univ Sch Med, Winston Salem, NC; 2) Dept Biochemistry, Wake Forest Univ Sch Med, Winston-Salem, NC; 3) Dept Internal Medicine, Wake Forest Univ Sch Med, Winston-Salem, NC; 4) Dept Public Health Sciences, Wake Forest Univ Sch Med, Winston-Salem, NC; 5) Dept Preventative Med and Biometrics, Univ Colorado Health Science Ctr, Denver, CO; 6) Dept Medicine, Univ California at Los Angeles, Los Angeles, CA; 7) Dept Medicine, Univ Texas Health Science Ctr at San Antonio, San Antonio, TX.

Proopiomelanocortin (POMC) has been found to be associated with rare Mendelian forms of obesity in children. In linkage studies the POMC region has been linked to leptin levels in Caucasian, Mexican American, and African American families. POMC polymorphisms have not been investigated in detail for association with obesity in the general population. Five single nucleotide polymorphisms (SNPs) were genotyped on 1049 Hispanic and African American individuals in the IRAS Family Study and tested for association with multiple obesity quantitative traits. General and family based association analysis of the data was performed using General Estimating Equations (GEE1) and haplotype analysis was performed using the quantitative pedigree disequilibrium test (QPDT). Five SNPs were genotyped (G-3460C, C17T, G3473A, C3755T, and A7069G). Evidence of association was seen primarily in Hispanics. SNP C3755T was consistently associated with P-values ranging from 0.011-0.045 for association with BMI, waist, visceral adipose tissue (VAT), and subcutaneous adipose tissue (SAT). G-3460C, G3473A, and A7069G were also found to be associated, though modestly, with additional obesity measurements (P-value 0.025-0.04). Results of haplotype analysis were consistent with single SNP analysis, with haplotypes containing C3755T showing the greatest evidence of association (P values ranging 0.024-0.048) for obesity traits. POMC polymorphisms show modest, but consistent evidence for association with obesity traits, consistent with the hypothesis that POMC contributes genetically to the development of obesity. Further studies will be necessary to clarify whether these or other SNPs are functional polymorphisms.

Genetic analysis of candidate modifier polymorphisms in α^0 thalassemia/HbE patients. *J.L. Whitacre¹, O. Sripichai^{1,2}, P. Winichagoon², K. Abel¹, A. Braun¹, S. Fucharoen².* 1) Sequenom, Inc., San Diego, CA; 2) Mahidol University, Nakornpathom, Thailand.

α^0 -thalassemia/HbE disease is one of the most common thalassemias in Southeast Asia and represents the main cause of childhood chronic disease. Recent worldwide migration has resulted in the appearance of abnormal hemoglobin alleles and associated disorders in other regions. Patients compound heterozygous for HbE and α^0 mutant alleles display remarkable variability in clinical expression, ranging from nearly asymptomatic to severe, transfusion-dependent disease clinically identical to homozygous α^0 -thalassemia. Given this clinical heterogeneity, additional genetic factors modifying disease severity remain to be discovered, themselves representing diagnostic targets for identifying at-risk individuals, or their products representing novel therapeutic targets.

Using an automated, chip-based platform for genetic analysis based on mass spectrometry (MassARRAY), we are examining to what extent genetic variation within the beta globin gene cluster contributes to disease severity in Thai/Chinese patients with α^0 thalassemia/HbE disease. A map of ~75 single nucleotide polymorphisms (SNPs) has been constructed spanning more than 80 Kb, including the locus control region (LCR) and all beta-like genes. These SNPs were identified through resequencing and from the public domain, including well-characterized RFLPs used in prior haplotype studies. Also included are assays for polymorphic sites reported to influence globin gene expression, specifically *Xmn-I* and the BP-1 binding sites upstream of α^G - and α -globin genes, respectively. Genotyping of other candidate modifier loci, including SNPs in genes encoding alpha hemoglobin stabilizing protein (AHSP), and the α -globin gene repressor BP-1, are underway. To date, almost 1250 patient DNAs have been collected and are being genotyped for the described assays. To identify additional modifier loci, carefully selected patient sub-groups representing the extremes in disease severity have been selected for DNA pool construction to be used in a genome-wide association study involving up to 100,000 gene-based SNPs.

Serotonin transporter SLC6A4 long promoter allele associated with suicide attempt in adults with a history of childhood-onset mood disorder. *N. Bulgin¹, J. Strauss¹, S. Shaikh¹, N. King¹, D. Goldman², X. Hu², C. George³, M. Kovacs³, J.L. Kennedy¹.* 1) Neurogenetics, Centre for Addiction and Mental Health, Toronto, ON; 2) NIAAA/NIH, Rockville, MD; 3) Department of Psychiatry, University of Pittsburgh School of Medicine, Western Psychiatric Institute and Clinic, Pittsburgh, PA.

Biological studies of suicide victims indicate altered central serotonergic function. However, results of serotonin system candidate gene studies have been less than consistent, especially those examining the serotonin transporter (SLC6A4) gene. Here we present the results of a unique genetic study examining differences in allele and genotype frequencies of the SLC6A4 gene variants between suicide attempters and non-attempters with a history of childhood-onset mood disorder (COMD). A total of 79 non-attempters and 97 attempters were genotyped for the intron 2 VNTR, the promoter insertion/deletion polymorphism (5-HTTLPR), and the long (l) G SNP described by Goldman et al. (2004). lG alleles were counted as s alleles to remain consistent with functionality. We first tested the sample for significant differences between attempters and non-attempters based on sex, affective disorder subtype, lifetime and current alcohol abuse/alcohol abuse or dependency (AD/AAD), and lifetime and current drug abuse. A history of at least one suicide attempt was significantly higher in females, those with a lifetime history of AD/AAD, and lifetime drug abuse ($p=0.04$, 0.01 , and 0.004 respectively). Intron 2 VNTR genotype frequencies did not differ significantly between the two groups when considering any of the covariates ($p>0.05$). In contrast, suicide attempters had a significantly higher frequency of the l/l 5-HTTLPR genotype than the l/s or s/s genotypes combined in both sexes (males: $p=0.02$; females: $p=0.03$), but not in those with lifetime drug abuse ($p>0.05$). Suicide attempt was significantly associated with the l/l genotype for AD/AAD subjects ($p=0.004$), but not for non-AD/AAD subjects ($p=0.21$). These results suggest that the l/l SLC6A4 genotype, in combination with AD/AAD, may have predisposed adults with a history of COMD to attempt suicide in our sample.

Structured association case-control and family-based association study on ADH4 gene and alcohol dependence.

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Foroud et al. (2003) reported that alcohol dehydrogenase 4 (ADH4) gene might be a risk factor for alcohol dependence (AD) in European-Americans (EAs). We investigated the relationship between ADH4 gene and AD in additional populations, including EAs and African-Americans. Seven SNPs spanning the ADH4 gene were genotyped in 365 healthy controls, 432 AD cases, and 124 small families with AD offspring. Comparing cases and controls within populations, we found significant differences in genotype frequencies for four of the seven ADH4 markers (adjusted global $p=0.004$). To evaluate the effects of possible population admixture on these positive associations, we examined the ancestry proportions of each subject through the program STRUCTURE, using independent and highly ancestry-informative markers including 37 STR markers, FY, and one ADH4 marker (rs1984362). These ancestry proportions were then input into the STRAT program to run a structured association analysis, which suggested that the genotypes of six of the seven ADH4 markers were associated with AD after controlling for population admixture effects ($p=0.011-0.047$). To further control for potential confounding of associations by sex, and to explore more models including the interactions among markers, a backward stepwise logistic regression analysis was employed, with diagnosis as the dependent variable, and the ancestry proportion, sex, and either ADH4 allele, genotype, haplotype probability or diplotype probability as the independent variables. This analysis resulted in the C/C genotype for the ADH4 promoter SNP (rs1800759, $p=0.013$) and the homozygous seven-variant diplotype (CCGATCG/CCGATCG, $p=0.049$) being significantly associated with AD. TDT analysis for the independent family sample showed that the C allele of SNP hcv2033010 ($p=0.036$) and the seven-variant haplotype CCGATCG ($p=0.018$) were overtransmitted with AD. Our evidence confirms that the ADH4 gene is a susceptibility gene for AD. (Foroud et al. Am J Hum Genet. 2003;73(5 suppl.):#2066).

Genome-wide expression analysis in a mouse model of chronic inflammatory bowel disease. *D. Brudzewsky*¹, *L.A. Larsen*¹, *M. Claesson*², *M. Gad*², *N.N. Kristensen*², *S. Knudsen*³, *Z. Tmer*¹. 1) Wilhelm Johannsen Centre for Functional Genome Research, IMBG, The Panum Institute, University of Copenhagen (Denmark); 2) MAI, The Panum Institute, University of Copenhagen (Denmark); 3) CBS, Biocentrum, Technical University of Denmark.

Chronic inflammatory bowel disease (IBD) is a multifactorial disorder with two major clinical forms, Crohns disease (MIM 266600) and ulcerative colitis (MIM 191390) with unknown etiology. In order to investigate the gene expression levels in the T cell transfer SCID mouse model for colitis, we have screened Affymetrix GeneChips (MOE430A, containing 22600 genes, ESTs and non-ESTs) using colon cDNA prior to and after induction of colon inflammation. Based on histology, weight loss and results from a CD4⁺ T-cell anti-entero antigen proliferation assay the mice were categorised into a non-transplanted control group and two inflammatory groups with low and high levels of inflammation, respectively. Hierarchal clustering of the gene expression results supported this categorisation of the mice. We observed that ~40% of the 200 genes with most significant changes in expression are down-regulated. Two genes of interest were *15-Pghd* and *Gelsolin*. *15-PGHD* (-3.1 fold; p=3.3e-06) and has previously been found to be down-regulated during lipopolysaccharide-induced fever and systematic inflammation. The *Gelsolin* gene (-2.3 fold; p=1.4e-05) has been suggested to contribute to the pathophysiology of microvascular dysfunction during pulmonary inflammation when plasma Gelsolin is depleted. Among the up-regulated genes were the *S100a8* gene (78.1 fold; p=6.12e-07) and the *S100a9* gene (138.0 fold; p=6.29e-06) which together with the chemokines *Cxcl-5* (52.8 fold; p=2.6e-05) and *Cxcl-1* (8.2 fold, p=9.6e-06) are known to be involved in trafficking of inflammatory cells from the blood into the inflamed tissues. These results indicate that the SCID mouse model of colitis displays a gene expression profile, in accordance with conditions for chronic inflammation. This model can therefore be useful in identifying genes, pathways and perhaps new therapeutics involved and useful in IBD.

Quantification of the cystatin B gene expression pattern by real-time PCR. *M. Kuronen*¹, *T. Joensuu*¹, *L. Huopaniemi*², *A.-E. Lehesjoki*¹. 1) Folkhälsan Institute of Genetics, Department of Medical Genetics and Neuroscience Center, Biomedicum Helsinki, University of Helsinki, Finland; 2) Rational Drug Design Program, Biomedicum Helsinki, Finland.

Unverricht-Lundborg disease (EPM1, OMIM254800) is an autosomal recessive inherited disorder characterized by onset at the age of 6-15 years, severe stimulus-sensitive progressive myoclonus and tonic-clonic epileptic seizures. The mutated gene responsible for the EPM1 phenotype, cystatin B (*CSTB*), encodes a cysteine protease inhibitor of the cystatin family. The most common EPM1-associated mutation is an unstable expansion of a 12-mer repeat unit located in the promoter region of *CSTB*. Three of the other six known mutations affect conserved splice site sequences and predict severe splicing defects. Two mutations predict a truncated protein through creating a premature termination codon or causing a frameshift, while one is a missense mutation replacing a highly conserved glycine to arginine in exon 1. Expression of the *CSTB* mRNA was quantified in several human tissues, and in patients with different EPM1-associated mutations using TaqMan real-time quantitative PCR and comparative C_T method. Prior to the TaqMan-analysis RNA from control, patient and carrier lymphoblastoid cells were isolated and reverse transcribed. Human TATA binding protein mRNA was used as the endogenous control. The results of the EPM1-patients and mutation carriers were compared to three unaffected controls. *CSTB* mRNA is ubiquitously expressed in both fetal and adult human tissues having the greatest expression in the lung. Comparable with results in Northern analysis of lymphoblast cell RNA, *CSTB* was markedly reduced in the EPM1 patients homozygous for the expansion mutation, showing 3-8% of the expression of the unaffected control. Patients compound heterozygous for the repeat expansion and either a splice site mutation or a nonsense mutation also show reduced *CSTB* expression.

Social Supports and Maltreatment History Co-Moderate Expression of Serotonin Transporter Gene on Childhood Depression: Demonstration of Gene and Complex Environments Interaction. *B.Z. Yang¹, H. Douglas-Palumberi¹, S. Houshyar², D. Lipschitz¹, J.H. Krystal^{1,3}, J. Gelernter^{1,3}, J. Kaufman¹.* 1) Dept Psychiatry, Yale Univ Sch Medicine, West Haven, CT; 2) Dept Psychology, Yale Univ; 3) Natl Center, PTSD Res, VA, CT, West Haven, CT.

We investigated how complex environmental factors, such as social supports and maltreatment history, co-moderate the effects 5-HTTLPR, a functional polymorphism at the serotonin transporter protein gene locus (SLC6A4), on the development of childhood depression. Participants included 101 children: 57 maltreated children who were removed from their parents homes due to allegations of abuse or neglect, and 44 demographically-matched community-control (CC) children who had no prior history of maltreatment or exposure to domestic violence. These 101 children were from 67 families with various numbers of sibs and half-sibs in each family. 5-HTTLPR and 35 STR markers, previously shown to differentiate American populations effectively, were genotyped. Because of sibling relationships, the subjects were not necessarily genetically independent. We ran STRUCTURE (Pritchard et al 2000) multiple times (to cluster population groups empirically and infer ancestry) such that each STRUCTURE run included only one child from each family. The average ancestral proportions (AP) for each subject were defined as the mean of the APs from the STRUCTURE outputs. Generalized Estimating Equations (GEE) were used to model the effects of risk or protective factors while handling familial correlations between subjects resulting from the inclusion of siblings in the sample. Age, sex, and AP scores were entered in all the models as covariates. The main effects of 5-HTTLPR genotype (s/s; l/s; l/l), group (maltreatment; community control), and the social support indices were explored, together with all possible two-way and three-way interactions. The results showed that all the main effects, some two-way and three-way interactions were significant ($p < 0.04$ to < 0.0001). We conclude that maltreatment history and level of social support co-moderate the effect of 5-HTTLPR on development of depression in maltreated children.

Evidence for association of a *CBLB* variant with Type 1 Diabetes and genetic interaction with *CTLA4*. R.

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Background and Aim. Due to an inhibitory role of Cbl-b in T-cell stimulation, dysregulation of Cbl-b may contribute to autoimmune diseases, including Type 1 Diabetes (T1D). The aim of the present study was to screen the human *CBLB* gene for mutations of importance in autoimmune disease and test for association to T1D and interaction with *CTLA4*.

Material and Methods. DNA from two collections of Danish T1D families, 1) 253 families and 2) 227 families, was used (Material 2 only for replication). Mutational screening, by sequencing, was performed in a subset of 24 individuals with T1D and at least one other autoimmune disease, as well as five controls without any autoimmune disease. Coding regions of the *CBLB* gene (Chr. 3q11-13.1) were screened. Genotyping was performed by RFLP-PCR, mutagenically separated PCR or primer extension method. SNPs were tested for association to T1D by Sib-transmission-disequilibrium test. To evaluate the degree of linkage disequilibrium (LD) in the region, the pairwise LD between the SNPs in *CBLB* was evaluated.

Results. Identified SNPs were genotyped in a T1D family material and tested for T1D association. A rare SNP in exon 12 (MAF:0.02) was associated to T1D. Replication of this observation in another Danish T1D family material was attempted, but did not reach significance. However, no difference existed between the two data sets, which were then pooled. When 480 families were evaluated significant association to T1D was still observed. Stratifying *CBLB* exon 12 data, according to genotypes of an established *CTLA4* SNP (*CT60*), strengthened T1D association in the *CT60* high risk subgroup. A high degree of LD in the region was demonstrated, three LD blocks included most of the gene.

Conclusion. We demonstrated T1D association of a rare polymorphism in exon 12 of the human *CBLB* gene. Gene-gene interactions probably play substantial roles in T1D susceptibility and evidence for genetic interaction between the *CTLA4* and *CBLB* genes, both involved in T-cell activation, was observed.

Tyrosinase is a phenotypic modifier of primary congenital glaucoma in Saudi Arabia. C. Bidinost¹, N. Hernandez^{1,2}, D. Edward³, A. Al-Rajhi⁴, J.R. Lupski⁵, R.A. Lewis⁵, B.A. Bejjani^{1,2}, D.W. Stockton⁵. 1) Washington State University, Spokane; 2) Sacred Heart Medical Center, Spokane, WA; 3) University of Illinois in Chicago; 4) King Khaled Eye Specialist Hospital, Riyadh, Saudi Arabia; 5) Baylor College of Medicine, Houston, TX.

Autosomal recessive primary congenital glaucoma (PCG) shows incomplete penetrance in the Saudi Arabian population. About 94% of individuals with PCG in Saudi Arabia have identifiable homozygous or compound heterozygous mutations in cytochrome P4501B1 (CYP1B1). We performed a genome-wide screen for modifier loci responsible for the incomplete penetrance, with both parametric and non-parametric analyses, which was unproductive. Recently, tyrosinase (*Tyr*) was found to modify the drainage structure phenotype in *Cyp1b1*^{-/-} mice, where *Tyr* deficiency caused more severe ocular dysgenesis. To test a possible modifying role for TYR on PCG in the Saudi population, we sequenced all five *TYR* coding exons in 60 individuals with homozygous and compound heterozygous *CYP1B1* mutations from Saudi families with incomplete penetrance, 50 unaffected Saudi controls, 50 unaffected non-Saudis, and 50 PCG-affected Saudis with homozygous and compound heterozygous *CYP1B1* mutations from fully penetrant families. We identified one polymorphism in exon 1 (575CA, S192Y) and a mutation in exon 4 (1205GA, R402Q) that results in a temperature-sensitive enzyme, TYR(R402Q). The exon 1 polymorphism showed similar distribution in all groups tested, but the frequency of heterozygosity for TYR(R402Q) is significantly higher in the fully penetrant families than in the nonpenetrant families ($p < 0.001$). There is no significant difference in the distribution of TYR(R402Q) between affected individuals and their nonpenetrant relatives. The heterozygotes for the R402Q mutation who have homozygous or compound heterozygous *CYP1B1* mutations have an 18-fold relative risk for PCG compared to individuals with an identical CYP1B1 genotype without the R402Q mutation. These data suggest that TYR(R402Q) is a susceptibility or liability allele for PCG in at-risk individuals with *CYP1B1* mutations in the Saudi population.

DIAPH2 is a susceptibility gene for POF. *S. Bione*¹, *C. Sala*², *F. Rizzolio*², *M. Goegan*², *R. Ricotti*¹, *A. Marozzi*³, *W. Vegetti*⁴, *R. Nappi*⁵, *D. Toniolo*^{1,2}. 1) IGM-CNR, Pavia, Italy; 2) DIBIT-HSR, milano, Italy; 3) Dept of Biology and Medical Sciences, University of Milano, Italy; 4) Dept of Obstetrics and Gynaecology, University of Milano, Italy; 5) Dept of Obstetrics and Gynaecology, University of Pavia, Italy.

Premature Ovarian Failure (POF) is a disorder characterized by lack of ovulation and elevated gonadotropin level before 40 years of age. POF has a frequency of about 1% among females, but with the increase of women reproductive age it has become a relevant cause of infertility. A genetic component of the disorder is demonstrated by the frequent finding of POF familial cases, but few responsible genes were identified accounting for a small proportion of cases. The DIAPH2 gene in Xq22 was proposed as a candidate gene for POF as it was found interrupted by the breakpoint of a balanced X/autosome translocation carried by a familial case of POF. Moreover the DIAPH2 gene is one of the two human homologue of the *Drosophila melanogaster* dia gene whose mutated alleles affect fruit fly fertility. To determine the contribution of the DIAPH2 gene in the pathogenesis of POF, we performed a case-control study on a panel of 248 Italian POF patients and 404 matched controls to identify allelic variants associated with the disorder. More than 30 single nucleotide polymorphisms (SNPs) spanning a region of about 1.5 Mb containing the DIAPH2 gene were genotyped and the haplotypic structure of the region was constructed using a pair-wise maximum likelihood estimation method. The region resulted split into three linkage disequilibrium (LD) blocks of about 540, 340 and 79 kb in length. One of the SNP analysed (L1) showed significant association to the POF condition ($p=0.0187$). The genotypes at the L1 locus resulted significantly different between patients and controls ($p=0.0053$) showing an excess of heterozygous individuals. The results can be explained by a reduction of male and homozygous female fertility. Experiments are in progress to verify the hypothesis. Our results demonstrated the involvement of DIAPH2 as a susceptibility gene for POF and provide new evidences in favour of a multifactorial pattern of inheritance of X-linked POF.

Identification and characterization of a Xp22.33 : Yp11.2 translocation causing a triplication of several genes of the PAR1 region in a XX male patient with severe Systemic Lupus Erythematosus. *P. Chagnon*¹, *R. Schneider*², *J. Hebert*^{1, 3}, *P. Fortin*⁴, *S. Provost*¹, *C. Belisle*¹, *V. Bolduc*¹, *M. Gingras*¹, *M. Lussier*⁵, *E. Silverman*², *L. Busque*¹. 1) Research Centre, Maisonneuve-Rosemont Hospital, University of Montreal, Montreal, Canada; 2) The Hospital for Sick Children, University of Toronto, Toronto, Canada; 3) The Leukemic Cell Bank of Quebec (BCLQ), Maisonneuve-Rosemont Hospital, Montreal, Canada; 4) University Health Network, Toronto, Canada; 5) HemaX Genome inc., Montreal, Canada.

The X;Y translocation breakpoint sequence of a XX male patient suffering from prepubertal Systemic Lupus Erythematosus (SLE) was characterized with the intention of identifying predisposing gene(s) for SLE. Spectral Karyotyping (SKY) of the patients metaphase chromosomes showed normal autosomes and two X chromosomes with one displaying a small portion of the Y chromosome. Fluorescence in situ hybridization (FISH) studies using a SRY gene-specific probe revealed that the breakpoint on the Y chromosome occurred downstream of this gene. Using a Y-chromosome PCR walking strategy and inverse PCR, we showed that the abnormal recombination occurred between retroviral long terminal repeats (LTR) of the pseudoautosomal regions of Xp22.33 (position 0.95 mb) and Yp11.2 (4.20 mb). The complete sequence of the breakpoint was determined. The recombination event caused a partial duplication of the pseudoautosomal region 1 (PAR1) in the derivative X chromosome. As a result, a portion of the genes of the PAR1 are found in three copies (partial trisomy) in the patients genomic DNA, which was confirmed by real time quantitative PCR. Gene expression analyses using TaqMan Assay also revealed an overexpression profile in the patient compared to normal individuals. The uniqueness of the translocation, the rarity of male severe prepubertal SLE, and the increased incidence of SLE in Klinefelter syndrome, who also have a triplication of the PAR1 region, point to a relationship between the partial triplication of the PAR1 region and SLE development in the patient. There is major interest to verify if one or more of the triplicated genes are involved in common SLE or other autoimmune disorders.

Association of the 5-*HTR1A* gene region with Autism Spectrum Disorders. C. Correia¹, A.M. Coutinho¹, C. Bento², C. Marques², A. Ataíde², T. Miguel², L. Borges², G. Oliveira², A.M. Vicente¹. 1) Instituto Gulbenkian de Ciência, Oeiras, Portugal; 2) Hospital Pediátrico de Coimbra, Coimbra, Portugal.

High levels of platelet serotonin have been consistently found in a proportion of autistic patients, suggesting that an abnormality in the serotonin (5-HT) system may be involved in autism etiology. We have previously found that 5-HT transporter gene variants are associated with an increased risk for hyperserotonemia. However, the reported association only accounts for a proportion of the total 5-HT variance. Other genes and environmental factors are likely involved in hyperserotonemia, and the somatodendritic autoreceptor 5-HT_{1A} is a strong candidate. In this study, we examined the association with autism and serotonin levels of two microsatellites in the 5-*HTR1A* gene region, D5S668 and D5S1956, in a sample of 221 autistic nuclear families. We found strong transmission disequilibrium of alleles at the D5S668 marker (Allele-wise TDT: $\chi^2=30.13$; $df=9$; $P=0.0004$), with preferential transmission of allele 1 ($\chi^2=6.898$; $P=0.0087$) and less frequent transmission of allele 2 ($\chi^2=3.903$; $P=0.048$). We examined the data for parent-of-origin effect and found significant results for paternal transmissions for D5S668 (Allele-wise TDT: $P=0.005$), which is suggestive of maternal imprinting. No transmission disequilibrium of D5S1956 was observed. In 144 trios, no significant association of serotonin levels was found with either marker, although a trend was found for D5S668 ($P=0.0596$ for D5S668; $P=0.0854$ for D5S1956). A larger sample size is necessary to confirm a possible role in the determination of serotonin levels. In conclusion, we obtained strong evidence for the existence of a susceptibility gene for autism in the region of marker D5S668, which might also be implicated in the regulation of serotonin levels. Further studies are being carried out with SNPs in the 5'UTR of 5-*HTR1A* gene to test our hypothesis that this gene is responsible for the observed association with autism and to clarify its possible role in the determination of serotonin levels.

Association of the DTNBP1 locus with schizophrenia in a US population. *B. Funke¹, C.T. Finn¹, A.M. Plocik¹, S. Lake², P. DeRosse³, J.M. Kane³, R. Kucherlapati¹, A.K. Malhotra³.* 1) Harvard Partners Center for Genetics and Genomics, Boston, MA; 2) Channing Laboratory, Brigham and Women's Hospital, Boston, MA; 3) Psychiatry Research, The Zucker Hillside Hospital, Glen Oaks, NY.

Linkage and association studies have recently implicated the dystrobrevin-binding protein 1 (DTNBP1) in the etiology of schizophrenia. We analyzed seven previously tested DTNBP1 single nucleotide polymorphisms in a cohort of 563 cases with schizophrenia or schizoaffective disorder and 649 control subjects. The minor alleles of three SNPs (P1578, P1763, P1765) were positively associated with the diagnosis of schizophrenia or schizoaffective disorder in the Caucasian subset of the study cohort (258 cases; 467 controls) with P1578 showing the most significant association ($p = 0.0026$). The same three SNPs were also associated in a smaller Hispanic subset (51 cases; 32 controls). No association was observed in the African American cohort (215 cases; 74 controls). A stratified analysis including the Caucasian and Hispanic subsets showed association with the minor alleles of four SNPs (P1578, P1763, P1320 and P1765). Again, the most significant association was observed for P1578 ($p = 0.0006$). Haplotype analysis supported these findings with a single risk haplotype significantly overrepresented in the Caucasian sample ($p = 0.005$). Our study provides further evidence for a role of the DTNBP1 gene in the genetic etiology of schizophrenia.

Narrowing down the haplotype association for preeclampsia on chromosome 2p25. *H. Laivuori*¹, *E. Kerkelä*², *H. Peterson*², *H. Jiao*², *K. Kivinen*², *V.-V. Mäkelä*², *R. Kaaja*³, *O. Ylikorkala*³, *J. Kere*². 1) HUSLAB Department of Clinical Genetics, Helsinki University Central Hospital, Helsinki, Finland; 2) Department of Biosciences at Novum, Karolinska Institutet, Huddinge, Sweden; 3) Department of Obstetrics and Gynecology, Helsinki University Central Hospital, Helsinki, Finland.

Preeclampsia, a potentially life-threatening disease during pregnancy, is characterized by hypertension and proteinuria. The epidemiology of preeclampsia suggests a genetic basis for the disorder. We have previously mapped a susceptibility locus for preeclampsia on chromosome 2p25 in 15 Finnish families, and the linkage of the previously mapped locus was verified with additional microsatellites (NPL score = 4.09, $p = 0.00036$). We continued finemapping using high-density single nucleotide (SNP) polymorphism genotyping. Further analysis of SNPs within the peak region revealed a significant association ($p = 0.0099$), and the region was narrowed down to 290 kb by a 3-SNP haplotype. Sequencing analysis of a 80-kb area around the peak marker in one woman homozygous for the haplotype yielded 57 SNPs. Twenty of the those SNPs were chosen (about 4 kb interval) for genotyping in all of the studied individuals, and the association was confirmed ($p = 0.0287$). The new peak region was centralized to eight SNPs with a haplotype (22112*11) showing more often in the affecteds than in the non-affecteds (22% vs. 9% of frequencies). The remaining SNPs found 13 kb upstream and 8kb downstream from the two peak markers will be genotyped for the final genetic dissection of the whole putative region.

Family-based and case-control association of Brain-Derived Neurotrophic Factor (BDNF) and adult ADHD. *M.B. Lanktree, P. Muglia, A. Squassina, M. Krinsky, U. Jain, F.M. Macciardi, J.L. Kennedy.* Centre for Addiction and Mental Health, Clarke Site, Toronto, ON, Canada.

Introduction: Studies have shown that adult ADHD has an increased prevalence in families, possibly due to a greater genetic liability. Neuroimaging, pharmacological data, and candidate-gene association studies indicate that the dopamine system plays a crucial role in the pathophysiology of ADHD. The Brain-Derived Neurotrophic Factor (BDNF) is a neurotrophin involved in survival, differentiation, and synaptic plasticity of several neuronal systems including the dopaminergic system and is expressed throughout the life of the neurons. **Methods:** In the present study we tested the association between six BDNF polymorphisms (five SNPs and one STR) and adult ADHD. The sample consisted of 119 trios comprised of an adult ADHD proband and their biological parents and an independent sample of 121 ADHD cases and a corresponding number of sex, age and ethnically matched controls. Association was tested using Haplotype Relative Risk (HRR), Haplotype Trend Regression (HTR) and quantitative tests using ANOVA. **Results:** Association was found at each marker ($p < .05$) with the strongest linkage at the STR ($p < .005$). Quantitative trait analysis was performed comparing core symptom Wender and BADDs mean scores across genotype groups. No significant differences were found in symptom scores between the genotype groups within cases. Subjects who met the criteria for depression were removed from the study, however, we examined the affective symptom scores on the BADDs scale to determine if our positive results were artifacts of comorbid depression and no association was found. Two common haplotypes were found and Haplotype Trend Regression (HTR) analysis of the six polymorphisms showed one was a risk haplotype and one was a protective haplotype ($p < 0.005$). **Conclusions:** While BDNF has been strongly implicated in affective disorders, no previous studies have investigated for the presence of association between ADHD and BDNF. Our results suggest that the BDNF gene may play a role in increasing the susceptibility to ADHD.

Association of a COMT promoter polymorphism with schizophrenia. *A.K. Malhotra³, C.T. Finn¹, A.M. Plocik¹, S. Lake², P. DeRosse³, J.M. Kane³, R. Kucherlapati¹, B. Funke¹.* 1) Harvard Partners Center for Genetics and Genomics, Boston, MA; 2) Channing Laboratory, Brigham and Women's Hospital, Boston, MA; 3) Psychiatry Research, The Zucker Hillside Hospital, Glen Oaks, NY.

Positional as well as functional evidence implicates the COMT gene in the genetic etiology of several psychiatric disorders, but results from previous association studies have been inconsistent. We genotyped four known COMT single nucleotide polymorphisms (SNPs) in 717 total cases (schizophrenia n=452, schizoaffective disorder n=111, mood disorder n=154) and 649 controls. The SNPs included the Val108/158Met polymorphism, which affects COMT activity and SNPs rs737865 and rs165599, which were highly associated in a previous study (1). In addition, we genotyped a SNP in the P2 promoter (-278A/G; rs2097603) as recent evidence suggests that cis-acting variation in the COMT gene may confer susceptibility to schizophrenia (2). In the Caucasian group including all affecteds (446 cases, 467 controls) the A allele of rs2097603 as well as the G alleles of the Val/Met polymorphism and rs165599 were significantly associated (p=0.0079; p=0.0096; p=0.0167), and one haplotype consisting of the three positively associated alleles plus the G allele for rs737865 (A-G-G-G) showed a trend towards significance (p=0.06). When the single SNP analysis was limited to subjects with schizophrenia and schizoaffective disorder, the promoter polymorphism remained significant while the Val/Met variant showed a trend towards significance in females. In the remaining set of patients (mood disorder), the Val/Met SNP and rs165599 yielded significant p-values of < 0.05. While our results confirm a possible contribution of cis-acting COMT variation to schizophrenia, they do not clarify the role of the Val/Met variant, which may reflect the difficulty of standardizing assessment of patients with psychiatric disease in the absence of objective biomarkers. 1. Shifman et al. (2002) *Am J Hum Genet* 71: 1296; 2. Bray et al. (2003) *Am J Hum Genet* 73:152.

A Pharmacogenetic Study of Bipolar Disorder: The CREB Family of Genes. *F. Mamdani, A. Sequeira, M. Alda, P. Grof, G. Rouleau, G. Turecki.* Douglas Hospital Research Centre, McGill University, Montreal, Quebec, Canada.

Bipolar disorder (BD) is a severe psychiatric disorder that affects 1% of the population. The importance of genes in BD has been enforced through studies using families, twins and adopted children. Recently, there have been many attempts to identify specific genes that are involved in BD; however, the task of finding susceptibility genes is not easy because BD is a complex disorder. One way to go about finding these susceptibility genes is using a pharmacogenetic approach, which implies looking at genes that may be involved in drug response. Pharmacogenetics has been increasingly used in the past 5-10 years and provides hope that eventually drug response will be a predictable factor. The most commonly used mood stabilizer in the treatment of BD, with approximately, 60% of patients showing some form of response. Unfortunately, lithium's mechanism of action has still not been defined, but studies have been consistently suggesting that lithium may act by modulating signal transduction pathways, and particularly, the cAMP pathway and CREB, which regulates transcriptional activity. An association study was carried out looking at the CREB1, CREB2 and CREB3 genes, located at ch 2q32.3-q34, 22q13.1 and 9pter-p22.1, respectively. A total of four promoter single nucleotide polymorphisms (SNP), 5 SNPs in the UTR, 5 exonic and 6 intronic SNPs were investigated for their frequency and haplotype distribution in a BD sample of 185 lithium responders and 57 nonresponders and 127 controls using a SNaPshot multiplex reaction. Our results suggest that the CREB1-1 SNP (A to T change, $P = 0.055$) and the CREB2-2 SNP (A to G change, $P = 0.034$) may have an effect on BD and lithium response.

Associations with schizophrenia over a 365kb region at Chromosome 3q13. *M.E. Talkowski¹, K.V. Chowdari¹, H. Mansour¹, J. Wood¹, M. Owen², M. O'Donovan², N. Norton², V.L. Nimgaonkar¹.* 1) University of Pittsburgh, Pittsburgh, PA; 2) University of Wales College of Medicine, Cardiff, UK.

We report on case-control and family based analyses consistent with SNP based associations over a 1.3mb region flanking the dopamine D3 receptor gene (*DRD3*). A ser9gly coding variant at exon1 of *DRD3* has been investigated extensively, but consistent associations have not been detected. We have investigated 69 SNPs at *DRD3*, as well as flanking regions.

We initially genotyped 15 SNPs spanning a 365 kb region centered on *DRD3* among 331 patients with schizophrenia/schizo-affective disorder (SZ), including 151 cases with available parents (case-parent trios). Cord blood samples from local live births served as unrelated, unscreened controls (n = 279). Transmission Disequilibrium tests (TDT) revealed significant associations with 5 SNPs. Significant case-control associations were also detected at 5 SNPs spanning 365kb about the *DRD3* locus, with case-control frequency differences ranging from 4.7% to 11%. Linkage disequilibrium (LD) patterns suggest four or more distinct associated haplotype blocks in this region.

To rapidly evaluate the bounds of the associated region, we next estimated allele frequencies among samples of pooled DNA from cases and controls using a novel re-sequencing protocol (n=200 per pool). We thus investigated 54 additional SNPs spanning a 1.3 mb region. We detected frequency differences over 7% for 11 additional SNPs across this region (range 7%-21%). Similar case-control differences were detected for 7 of these SNPs in an independent sample from Cardiff, UK (n=540 cases, 360 controls; range 4%-14%). The differences are particularly noticeable centromeric to *DRD3*.

Our analyses using individual and pooled genotyping assays thus suggest that associations with SZ exist over an extensive genomic region at chromosome 3q13. Further replicate studies are ongoing.

Further refinement of the susceptibility locus for migraine with aura on 4q24. *P.J. Tikka¹, M.A. Kaunisto¹, G. Oswell², M. Kallela³, J.C. Papp⁴, A. Presson⁴, E. Hämäläinen¹, T. Hiekkalinna⁵, H. Havanka⁶, J. Terwilliger⁷, M. Färkkilä³, L. Peltonen⁵, M. Wessman^{1,8}, A. Palotie^{1,2}.* 1) Biomedicum Helsinki, Research Program in Molecular Medicine and The Finnish Genome Center and Dept. of Clinical Chemistry, University of Helsinki, Finland; 2) Dept. of Pathology and Human Genetics, David Geffen School of Medicine at UCLA, USA; 3) Dept. of Neurology, University of Helsinki, Finland; 4) Dept. of Statistics, David Geffen School of Medicine at UCLA, USA; 5) Dept. of Medical Genetics, University of Helsinki and Dept. of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 6) Dept. of Neurology, Länsi-Pohja Central Hospital, Kemi, Finland; 7) Columbia Genome Center, Columbia University, USA; 8) Folkhälsan Research Center, Helsinki, Finland.

Migraine is a complex neurovascular disease with a recurrent transient focal disabling headache as the main symptom. The neuronal dysfunction preceding the headache phase, which typically presents as visual symptoms, is the distinguishing feature in migraine with aura (MA). Migraine affects 10 - 15 % of adult females and 2 - 6 % of adult males, and 15 % of migraine attacks are of the MA type. Despite several genome-wide linkage and candidate gene studies predisposing genes for common types of migraine have not yet been identified. We have collected a large sample of Finnish MA families consisting of 600 families and more than 4000 DNAs. Using a subset of 50 families our group has reported linkage (HLOD 4.45) to 4q24, and subsequently restricted it to a 10 Mb (6.1 cM) region from D4S3006 to D4S1591. In order to further refine the position of an MA susceptibility locus, we performed fine mapping by genotyping 50 microsatellite markers and 110 SNPs. The -1 lod confidence interval highlighted the region between D4S1578 and D4S1572 as the most likely position. FBAT, Pseudomarker and Transmit analyses of 110 SNPs provided suggestive (p-values ranging from 0.0003 to 0.067), but not conclusive evidence for association in linked pedigrees. Further fine mapping using 400 additional SNPs and construction of combined microsatellite and SNP haplotypes is currently in progress.

Association of polymorphisms and haplotypes in the elastin gene in Dutch patients with sporadic aneurysmal subarachnoid hemorrhage. Z. Urban^{1, 2}, Y.M. Ruigrok³, S. Wolterink¹, S. Böhn^{1,2}, C. Wijmenga⁴, G.J.E. Rinkel³. 1) Biochemistry, JABSOM, Univ Hawaii, Honolulu, HI; 2) Pediatrics, Washington University Medical School, St. Louis, MO; 3) Neurology, University Medical Center, Utrecht, Netherlands; 4) Biomedical Genetics, University Medical Center, Utrecht, Netherlands.

A locus containing the elastin gene has been linked to familial intracranial aneurysms in two distinct populations. We investigated the association of single nucleotide polymorphisms (SNPs) and haplotypes of SNPs in the elastin gene with the occurrence of subarachnoid hemorrhage (SAH) from sporadic aneurysms in the Netherlands. We genotyped 167 SAH-patients and 167 matching controls for 18 exonic and intronic SNPs in the elastin gene. A Bonferroni correction was applied for multiple comparisons to all novel associations, with a correction factor derived from the number of SNPs tested (p_{corr}). There was a statistically significant association of SAH with a SNP in exon 22 of the elastin gene (minor allele frequency was 0.000 in patients and 0.028 in controls, OR 0.0, 95% CI 0.0-0.7, $p=0.004$; $p_{\text{corr}}=0.05$) and possibly with a SNP in intron 5 (minor allele frequency was 0.062 in patients and 0.128 in controls, OR 0.5, 95% CI 0.2-0.8, $p=0.007$; $p_{\text{corr}}=0.08$). Haplotypes of intron 5/exon 22 ($p_{\text{corr}}=0.002$), intron 4/exon 22 ($p_{\text{corr}}=0.02$), and intron 4/intron 5/exon 22 ($p=9.0 \times 10^{-9}$) were also associated with aneurysmal SAH. Variants and haplotypes within the elastin gene are associated with the risk of sporadic SAH in Dutch patients. Gradual increase of statistical power with the inclusion of 2 or 3 SNPs in the studied haplotypes supports the validity of our conclusion that the elastin gene is a susceptibility locus for SAH.

Obesity and genes of the dopamine system: A study of a Mexican population. *L. VanderBeek¹, M.B. Lanktree¹, R.D. Levitan¹, B. Camarena², H. Nicolini³, J.L. Kennedy¹*. 1) Centre for Addiction and Mental Health, Clarke Site, Toronto, ON, Canada; 2) National Institute of Psychiatry Ramón de la Fuente, Mexico City, Mexico; 3) Mexico City University, Mexico City, Mexico.

Introduction: We have recently reported a preliminary association between the 7-repeat allele of the dopamine-4 receptor gene and high lifetime body mass index in a Canadian sample of overeating women with Seasonal Affective Disorder (SAD). Particularly in high-risk populations, we speculate that dopamine genes may influence hedonic tone, establishing increased vulnerability to obesity in an environment where highly palatable, high caloric foods are plentiful. In the current study we examined whether genes coding for the dopamine D2 and D4 receptors (DRD2, DRD4) were associated with obesity in a sample of 44 small Mestizo families (people of mixed Spanish and Native Mexican ancestry) with an identified obese proband. **Methods:** The DRD4 VNTR and D2Taq1A polymorphisms were typed and quantitative measures were collected for all individuals (total N=123). ANOVA was used to compare quantitative measures in each of the genotypic groups using all individuals, while FBAT analysis was used to assess the transmission of quantitative phenotypes to affected probands. **Results:** In probands with obesity, FBAT found significant association between transmission of the 4-repeat allele of the DRD4 VNTR and increased BMI ($p=.04$). ANOVA analysis showed a non-significant trend associating the 4-repeat allele and high BMI ($p=.15$). These results were found with allele 4, as opposed to our prior finding in overeating women with SAD, in which the 7-repeat variant was the risk allele for obesity. **Conclusions:** The fact that different alleles of the DRD4 VNTR polymorphism may play a role in obesity in high risk Canadian vs. Mexican populations suggests that either the DRD4 VNTR polymorphism is in linkage disequilibrium with other genes contributing to obesity, or perhaps that different mechanisms are involved in obese patients where seasonality is or is not a strong environmental factor.

Functional polymorphisms of the human peroxisome proliferator-activated receptors gamma2 gene are associated to type 2 diabetes in African-Americans. *Q. Song¹, Q.W. Wang¹, Q. Xu¹, R. Kittles², G.H. Gibbons¹, C. Rotimi², The Africa America Diabetes Mellitus (AADM) Study.* 1) Cardiovascular Res Inst, Morehouse School of Medicine, 720 Westview Dr SW, Atlanta, GA 30310; 2) The National Human Genome Center, Howard University, Washington, DC 20059.

Peroxisome proliferator activated receptor gamma2 (PPAR γ 2) is a key transcription factor in adipocyte differentiation and lipid metabolism. No polymorphisms on this gene have been associated to diabetes in African-Americans. In this study, we systematically screened the 5-flanking region of human PPAR γ 2 gene and identified 11 novel single nucleotide polymorphisms (SNPs). Genetic association analysis in the Africa America Diabetes Mellitus (AADM) cohort showed that 2 SNPs, which are common in both African-Americans and Caucasians, are significantly associated with type 2 diabetes. Analysis of linkage disequilibrium (LD) and haplotype showed markedly divergent LD block structure, haplotype frequencies and single SNP frequencies among different ethnic US populations. These 2 common promoter SNPs are tightly in LD with Pro12Ala in Caucasians, but not in LD with Pro12Ala in African-Americans. Bioinformatic analysis using the VISTA program indicated that these 2 polymorphisms reside in an evolutionarily highly conserved region. Systematic functional analysis was performed by electrophoretic mobility shift assay (EMSA) and promoter-reporter assays. EMSA showed that these 2 SNP altered the DNA-protein binding intensities and may affect the recruitment of transcription factors during the PPAR γ 2 gene expression. Promoter deletion analysis showed that these 2 SNPs reside in regions that contain functionally important cis-elements of the PPAR γ 2 gene. Haplotype- and allele-specific PPAR γ 2 promoter reporter constructs showed that SNP-3 dramatically altered the PPAR γ 2 promoter activity in differentiated 3T3L1 adipocytes, and there is an interaction between alleles of SNP-3 and SNP-5 on their regulation of PPAR γ 2 transcription. Taken together, our data showed that there are functional-relevant genetic polymorphisms in the PPAR γ 2 promoter that are associated with the risk to develop type 2 diabetes.

Sensory deficiencies in a mouse model for Bardet-Biedl syndrome. A.J. Ross¹, H. May-Simera¹, J.P. Chapple², P.M. Munro², J. Hill¹, J.R. Lupski³, P.E. Scambler¹, N. Katsanis⁴, P.L. Beales¹. 1) Molecular Medicine Unit, Institute of Child Health, UCL, London, UK; 2) Department of Pathology, Institute of Ophthalmology, UCL, London, UK; 3) Dept of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 4) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD.

Bardet-Biedl syndrome (BBS) is a pleiotropic disorder with primary features that include age-related retinal dystrophy, obesity, polydactyly, renal dysplasia, reproductive tract abnormalities and cognitive impairment. It is genetically heterogeneous, with mutations identified in eight genes to date (*BBS1-8*). Recently there has been several lines of evidence to suggest that the BBS phenotype may result from a defect in cilia formation and/or function, or intraflagellar transport (IFT). Since, in addition to having visual impairments, 3% of BBS patients also have sensorineural deafness, together with our recent results of olfactory function showing that 47% of patients also manifest partial or complete anosmia, we were prompted to evaluate a mouse model of BBS for sensory deficiencies. We generated a mouse model null for the *Bbs6/Mkks* gene, which shows strong homology to the type II chaperonins. Null mice display age-related retinal degeneration that eventually leads to a complete loss of the photoreceptor layer. TUNEL staining showed that apoptosis occurs in the outer nuclear layer of the retina at ~2 weeks of age. Hearing was assessed using a 20KHz frequency generator and 31% of mice were identified to be impaired; olfactory function was tested by scoring the retrieval of buried food. We suggest that the sensory deficiencies displayed by *Bbs6*^{-/-} mice may result from ciliary dysfunction in sensory neurons and we present here data from structural analyses of cilia in the olfactory epithelia, cochlear hair cells and photoreceptors.

The role of NACP-Rep1 Polymorphic repeat in the regulation of -synuclein transcription. *O. Chiba-Falek*¹, *J.A. Kowalak*², *S.P. Markey*², *R.L. Nussbaum*¹. 1) GDRB/NHGRI/NIH, Bethesda, MD; 2) NIMH/NIH, Bethesda, MD.

Mutations and triplications of the α -synuclein gene have been implicated in familial Parkinson's disease (PD) while certain polymorphic alleles at a complex microsatellite repeat, NACP-Rep1, located ~10 kb upstream of the gene, have been associated with sporadic PD. Using luciferase reporter constructs, we showed previously that two domains flanking the repeat interact to enhance expression while the repeat acts as a negative modulator. Furthermore, we demonstrated that the expression levels among four NACP-Rep1 alleles of different lengths varied significantly, over a 3-fold range, in the neuroblastoma cells, SH-SY5Y. Subsequently, we showed previously that a NACP-Rep1 allele defined by length can differ in sequence due primarily to the intra-length variation of the TC-TA dinucleotides. However, analysis of two sequence variant alleles of size "1" using the luciferase reporter system showed only a slight expression difference between these 2 variants. This finding suggests that the length of the NACP-Rep1 allele, rather than minor sequence variation, plays the main role in the transcriptional regulation of α -synuclein expression. Next we studied the factors that bind to the NACP-Rep1 element and the region surround it. Proteins that bind to the NACP-Rep1 element were purified from SH-SY5Y nuclear extract and identified by mass spectrometry. One of the DNA-binding proteins detected is poly (ADP-ribosyl) transferase/polymerase (PARP). PARP was shown to play a role in MPTP-induced parkinsonism and dopaminergic neuronal cell death in a mouse model. Using electrophoresis mobility shift and super shift assay as well as chromatin immuno-precipitation assay we were able to further confirm the binding of PARP to the NACP-Rep1 element. Finally, we demonstrated the role of PARP in the regulation of α -synuclein expression and in modulating its promoter/enhancer activity. Given that even a two-fold increase in α -synuclein expression may, over many decades, cause PD, the association of different NACP-Rep1 alleles with PD may be a consequence of polymorphic differences in transcriptional regulation of α -synuclein expression resulting from different NACP-Rep1 alleles.

Friedreich ataxia in carriers of somatically unstable borderline GAA repeat alleles. *I. De Biase*¹, *R. Sharma*¹, *M. Gomez*¹, *M. Delatycki*², *T. Ashizawa*³, *S.I. Bidichandani*¹. 1) Biochemistry and Mol Biol, University of Oklahoma HSC, Oklahoma City, OK; 2) Murdoch Childrens Research Institute, University of Melbourne, Australia; 3) Neurology, University of Texas Medical Branch, Galveston, TX.

Friedreich ataxia (FRDA) is the most common recessive ataxia, typically caused by a large GAA repeat expansion within the first intron of the *FRDA* gene. Patients develop ataxia, hypertrophic cardiomyopathy and diabetes. Disease-associated repeats are reported to contain 66-1700 triplet-repeats. Rare alleles with 30-65 uninterrupted triplet-repeats, called premutations, do not normally cause disease, but may expand into the pathogenic range when transmitted from parent to child. However, given the rarity of alleles in this size range, the demarcation between normal and disease alleles remains poorly defined. We report two FRDA patients with delayed onset, hyperreflexia, and gradually progressive disease, who in addition to being heterozygous for large expansions, had alleles with 44 and 66 triplet-repeats, respectively. Using small pool PCR analysis of different tissue sources we demonstrate that these alleles, although not clearly in the disease range, were somatically unstable. As many as 15% (GAA-44) and 78% (GAA-66) of somatic cells contained alleles with >66 triplet-repeats, constituting a plausible mechanism for the atypically mild FRDA phenotype. In fact, 4.6% of somatic cells in the constitutive carrier of the GAA-66 allele contained >100 triplet repeats. The brother of the patient with the GAA-44 allele was found to carry a fully expanded allele and an allele with 37 triplet-repeats (GAA-37). Presently, in contrast to his sister, this subject does not show any clinical features of FRDA. By SP PCR, the GAA-37 allele showed no somatic variability. Our small pool PCR analysis of other carriers of borderline alleles indicates that the threshold for the initiation of somatic instability is between 40-44 uninterrupted GAA triplets. Our data indicate that somatic instability of borderline alleles (>40-44 uninterrupted triplets) may confer a risk of developing clinical disease by resulting in a significant proportion of cells bearing disease-causing expansions in pathologically affected tissues.

In vitro RNA interference against MHV-3 virus. *M.C. Grippo¹, T. Campos-Pereira¹, R. Gilioli², I. Lopes-Cendes¹.*
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RNA Interference (RNAi) has become a powerful used tool for the analysis of gene function and silencing mediated by double stranded RNA molecules. The first applications of the RNAi was demonstrated recently, including silencing of some viruses in vitro. The objective was to verify the efficiency of this new technique in the combat of the virus MHV-3 (hepatite murine type3). MHV belongs to the Coronavirus family, being the more frequently pathogen identified in mice used in experimental models. The Coronavirus is an enveloped virus with a large plus-stranded genome RNA. The MHV-3 codifies four structural proteins: protein S, which forms the petal-shaped spikes; protein M, which serves as a bridge between the nucleocapsid and the viral enveloped and the nucleocapsid protein (N) that wraps the viral genome into a long, helical nucleocapsid. This last one was choose, as our target to produce siRNAs molecules. Design of siRNAs was based on the sequence genome of MHV(GenBank AF 201929). We obtained 2 siRNAs, named 634 and 910, based in the position of the messenger RNA. A T7 promoter sequence was inserted upstream to the 21-mer MHV oligonucleotides to mallow in vitro Transcription. 1×10^4 L-929 cells, susceptible for MHV-3, were cultivated in 24 wells plates for 24 hours. 270 ng/well of each RNAs were tested for transfeccion with lipofectin. After transfection, cells were infected with 1 TCID₅₀ of the virus and observed in an interval of 24 to 48 hours, for the presence of citopatic effect through the formation of sincititions. We used three types of controls siRNA molecules: irrelevant, mutated and MEM-Eagle. The analysis of the results, clearly show reduction of the citopatic effect of the MHV-3 in the cellular cultures treated with siRNA910, while siRNA634 demonstrated not to have a significant effect. Molecular quantitative analysis for confirmation of the observed results is in progress and will be carried out by quantitative RT-PCR. Tests with siRNAs directed to other sequences of the MHV genome and in vivo experiments are also planned in order to better evaluate the potential use of RNAi in the treatment of MHV-3 viral infection.

Positional Candidate Gene Evaluation for Age-Related Maculopathy. *Y.P. Conley^{1,2}, D.E. Weeks², A. Thalamuthu², T.S. Mah³, R.E. Ferrell², M.B. Gorin^{2,3}.* 1) Health Promotion & Development, Univ Pittsburgh, Pittsburgh, PA; 2) Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 3) Ophthalmology, Univ Pittsburgh, Pittsburgh, PA.

Age-related maculopathy (ARM) is a leading cause of visual impairment in elderly Americans with the prevalence expected to increase by approximately 60% over the next 20 years. Well-recognized as a complex genetic disorder, ARM is a major public health concern of unknown pathogenesis. Through the use of microsatellite-based genome wide scans with small ARM families, we have found consistent linkage for 1q31, 10q26 and 17q25 within 530 families consisting of 736 affected sib pairs. We have initiated association studies for 15 candidate genes based upon their localization within these intervals and/or their implicated roles in pathways that have been implicated in ARM pathobiology. The selected candidate gene products have functions associated with inflammatory processes, cholesterol/lipoprotein metabolism, and basement membrane integrity. We have conducted both family-based and case/control (330 well-characterized, age appropriate, non-ARM controls) association studies. Preliminary data analyses indicate that one gene involved with basement membrane integrity (HEMICENTIN) and one gene involved with cholesterol/lipoprotein metabolism (VLDLR) are putatively associated with ARM status based on our preliminary analyses. Additional analyses including replicate testing and haplotype analyses are currently being conducted to confirm these findings.

QTLs that modify light-induced retinal damage in albino mice; evaluation of the arrestin gene (*Sag*) for one

QTL. *M. Danciger*¹, *D. Worrill*¹, *J. Lyon*¹, *C. Yamashita*², *H. Yang*³, *D.B. Farber*², *M.M. LaVail*³. 1) Loyola Marymount Univ, Los Angeles, CA; 2) JSEI, UCLA Schl of Medicine, Los Angeles, CA; 3) Beckman Vision Center, UCSF School of Medicine, San Francisco, CA.

In a previous study of C57BL/6J-c^{2J} (B6a) and BALB/c albino mice, we found that ~50% of the genetic resistance to light-induced retinal damage shown by B6a was due to a QTL on distal Chr 3. There is strong evidence that the B6a, Met450 variant of RPE65 is the resistant allele underlying the QTL. However, the NZW/LacJ mouse carries the Met450 allele but is susceptible to light insult. One explanation is that NZW has other sensitive gene alleles that negate the protection conferred by RPE65-Met450. The purpose of this work was to test the above hypothesis. Progeny of an F1 intercross between NZW and B6a aged to ~3 months were exposed to 3 weeks of constant light at an in-cage illuminance of 20-70 ft-c. After exposure, retinal damage was quantified in a single 1- retinal section by averaging 12 measurements of the thickness of the outer nuclear layer in the posterior-superior retina. DNAs of 156 F2s were genotyped with 77 dinucleotide repeat markers spanning the genome; selected markers were typed in another 45 F2s. Sequencing and Western blot were done by standard methods. There was no QTL at distal Chr 3. There were QTLs on Chrs 1 (two), 10, 13, 14 and 16. The QTLs on Chrs 1 and 10 matched QTLs found in previous age-related and light-induced retinal damage studies. Sequencing of the arrestin gene in the mid Chr 1 QTL revealed two variants. One of these was an A/G variant 2 bp upstream of the start of translation. Although this variant did not predict a difference in translation, levels of arrestin were tested in the two strains before and after light exposure with Western blot analysis. No significant difference between strains could be detected.

The absence of a QTL at distal Chr 3 supports the hypothesis that RPE65 is responsible for the Chr 3 QTL found previously as both the B6a and NZW strains carry RPE65 Met450. The matching of QTLs from this study with QTLs in other studies suggests some commonality in retinal damage pathways.

Apolipoprotein E Gene Polymorphisms in Japanese Patients with Open Angle Glaucoma. *F. Mabuchi¹, S. Tang², D. Ando², M. Yamakita², J. Wang², K. Kashiwagi¹, Z. Yamagata², H. Iijima¹.* 1) Department of Ophthalmology, School of Medicine, University of Yamanashi, Yamanashi, Japan; 2) Department of Health Sciences, School of Medicine, University of Yamanashi, Yamanashi, Japan.

Objective: To date, it has been reported that the apolipoprotein E (APOE) gene have a role in the etiology of open angle glaucoma (OAG), with the 4 alleles being a risk factor. However, not all APOE studies have found an association, and among these the associations differ. Additionally, there has been no study about the association between them in the Japanese population. The current study was conducted to investigate whether the APOE gene polymorphisms were associated with OAG in the Japanese population. **Methods:** Genomic DNA was examined from 310 unrelated patients with OAG and 179 unrelated controls in the Japanese population. The presence or absence of the diseases in all patients and controls was based on clinical examination and/or ophthalmic records. The APOE allele frequency (2, 3, and 4 alleles) was studied by PCR-RFLP analysis, and compared between OAG patients and controls. **Results:** The average ages in the OAG patients and controls were 64.3 13.5 and 65.6 11.6 years (mean SD) respectively. The APOE genotype frequencies in the controls and OAG patients were 0% and 0% for 2/2, 10.1% and 4.5% for 2/3, 68.7% and 83.5% for 3/3, 0% and 0.7% for 2/4, 21.2% and 11.3% for 3/4, and 0% and 0% for 4/4 respectively, and there was a significant difference in the APOE genotype frequencies between these groups ($P < 0.001$ Chi-square test). The frequency of the OAG patients with 4 allele was significantly lower than those of the controls with 4 allele ($P = 0.009$ Fisher exact test). **Conclusion:** Contrary to the previous studies, the association between the 4 allele of the APOE gene and a reduced risk of OAG was found in the Japanese population. Further studies in the different racial population are desirable to understand more fully the relationship between the APOE gene polymorphisms and OAG.

Interaction of cigarette smoking and APOE genotype in age-related macular degeneration. *E.A. Postel¹, W.K. Scott², Y.-T. Fan², A. Agarwal³, J.R. Gilbert², J.L. Haines⁴, M.A. Pericak-Vance².* 1) Dept Ophthalmology, Duke Eye Center, Durham, NC; 2) Ctr Human Genetics, Duke Univ Med Ctr, Durham, NC; 3) Dept Ophthalmology, Vanderbilt Univ Med Ctr, Nashville, TN; 4) Ctr Human Genetics Research, Vanderbilt Univ Med Ctr, Nashville, TN.

Age-related macular degeneration (AMD) is a leading cause of vision loss in older adults and is caused by a complex interplay of genetic and environmental factors. Two well-replicated risk factors for AMD are history of cigarette smoking and APOE genotype. In an ongoing genetic-epidemiologic study of AMD, we examined these factors in a data set of 305 cases with AMD (65% female, mean age 75.9) and 176 rigorously examined controls (61.7% female, mean age 66.7). Smoking history was determined by a self-administered questionnaire. Several measures of smoking, truncated at 10 years prior to study enrollment, were examined for association with AMD. Of these, lifetime history (ever/never), duration, intensity and pack-years of smoking were significantly associated with risk of AMD after adjusting for age and sex in a logistic regression model ($p < 0.0001$ to 0.0007). Consistent with previous data, APOE-2 was positively associated with AMD and APOE-4 was slightly negatively associated, although neither effect reached statistical significance. Effects of APOE alleles as well as smoking measures became substantially stronger when cases were restricted to neovascular AMD only, and the risk increase due to APOE-2 became statistically significant (OR=2.1, 95% CI 1.1-4.2, $p=0.03$). To examine a potential gene-environment interaction of APOE-2 carrier status and lifetime smoking, separate models were constructed in smokers and non-smokers. In smokers, APOE-2 was significantly associated with neovascular AMD (OR=2.8; 95% CI: 1.1-7.2, $p=0.03$), adjusting for age and sex. In non-smokers, no significant effect of APOE genotype was detected. The increase in the strength of association between APOE-2 and neovascular AMD in the presence of smoking supports a role of gene-environment interaction in AMD etiology.

Molecular and clinical characterization of two Chinese families with maternally inherited Leber hereditary optic neuropathy. *J. Qu¹, R. Li², Y. Hu^{1,3}, X. Zhou¹, L. Yang², Y. Tong¹, F. Lu¹, M.X. Guan^{1,2,3}.* 1) Phthalmology and Optometry, Wenzhou Medical College, Wenzhou, Zhejiang, China; 2) Division and Program in Human Genetics, Cincinnati Children's Hospital Medical center, Cincinnati, Ohio; 3) School of Life Sciences, Wenzhou Medical College, Wenzhou, China.

Leber hereditary optic neuropathy (LHON) is a mitochondrial disorder that preferentially causes blindness in young adult males. We report here the molecular and characterization of two Chinese pedigrees with maternally transmitted LHON. In particular, five affected male of 29 matrilineal relatives in a four generation Chinese family (Family I) revealed a variable severity of bilateral vision dysfunction and age-of-onset. Sequence analysis of the complete mitochondrial genome in two pedigrees revealed the presence of G11778A mutation in the ND4 gene and a number of other nucleotide changes. Of these, four and seven known variants in the polypeptide coding genes lead to the replacements of amino acids in the family I and II, respectively. Other variants include polymorphisms in the D-loop region, 12S rRNA, 16S rRNA and silence mutations in the protein coding genes. None of these mtDNA variants are evolutionarily conserved and implicated to have significantly functional consequence. However, the novel A4435G mutation in the tRNAMet gene was identified in the family II. This mutation locates in the conventional position 34 of this tRNA. This position is evolutionarily conserved and critical site for tRNA function. Thus, it is implicated that the novel A4435G mutation may act as a secondary factor affecting the phenotypic expression of the G11778A mutation. Furthermore, incomplete penetrance in these families indicates the involvement of modulatory factors including nuclear modified genes in the phenotypic expression of vision impairment associated with the G11778A mutation.

Distribution of p53 haplotypes in primary open angle and low tension glaucoma. *J.L. Wiggs¹, D. Figueiredo¹, L.R. Pasquale¹, R.R. Allingham², M. Hauser³, E.A. DelBono¹, L.M. Olson⁴, M. Pericak-Vance³, J.L. Haines⁴.* 1) Dept Ophthalmology, Harvard Medical Sch, MEEI, Boston, MA; 2) Dept Ophthalmology, Duke University Medical Center, Durham, NC; 3) Center for Human Genetics, Vanderbilt University School of Medicine, Nashville, TN; 4) Center for Human Genetics, Duke University Medical Center, Durham, NC.

Primary open angle glaucoma (POAG) and low tension glaucoma (LTG) are characterized by retinal ganglion cell death associated with apoptosis. Many proteins participate in regulation of apoptosis including p53. The p53 codon 72 polymorphism has been evaluated in a Chinese POAG population (Lin et al., 2002) and an Indian POAG cohort (Acharya et al., 2002), with conflicting results. A third study of POAG patients from the UK found a difference in p53 codon 72 haplotype distribution between POAG and controls (Ressiniotis et al., 2004). Altered expression of p53 in lymphocytes from patients with low tension glaucoma has been demonstrated suggesting that p53 may have a greater role in retinal ganglion cell degeneration in these patients. The purpose of this study is to determine the distribution of the of p53 haplotypes in a population of individuals with POAG (n = 173) and a second population of individuals with low tension glaucoma (n = 84). Patients with POAG had an elevation of intraocular pressure associated with optic nerve damage and characteristic visual field defects. Patients with LTG had optic nerve damage and visual field defects without an elevation of intraocular pressure. Patients and age-matched controls (n = 106) were genotyped for two p53 polymorphisms, a 16 base pair insertion in intron 3, and the C/G substitution in exon 4 that changes the Proline at codon 72 to an Arginine. We observed a significant difference in the p53 haplotype distribution between LTG patients and controls (p < 0.01), but not between POAG patients and controls, suggesting that p53 may contribute to retinal ganglion cell degeneration in LTG patients.

Polymorphisms in the estrogen receptor (ESR2) gene are associated with peak bone mineral density in Caucasian men and women. *S. Ichikawa¹, D.L. Koller², M.L. Johnson¹, D. Lai², M. Peacock¹, S.L. Hui¹, C.C. Johnston¹, T. Foroud², M.J. Econs^{1, 2}.* 1) Medicine, Indiana University School of Medicine, Indianapolis, IN; 2) Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN.

Osteoporosis is a common multifactorial disorder, characterized by fragility fracture due to reduced bone mass. A major determinant of osteoporotic fractures is peak bone mineral density (BMD) achieved during young adulthood. Recently, we reported several quantitative trait loci (QTLs) for peak BMD identified by 9-cM genome scan. Significant linkage for trochanter BMD in Caucasian and African American sister pairs was found at chromosome 14q (LOD score = 3.5), where the estrogen receptor (ESR2) gene is located. Estrogens are necessary for the acquisition of optimal peak bone mass, and functional sequence variations in ESR2 are likely to influence acquisition of peak bone mass. To determine whether ESR2 polymorphisms are associated with peak BMD in our population, we tested eleven single nucleotide polymorphisms (SNPs) encompassing the entire ESR2 gene. Peak BMD at femur and lumbar spine was measured in 190 men (age 20-61) and 588 women (age 21-52) from Indiana. SNP genotyping was performed using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry of allele-specific primer extension products. We found statistically significant association between ESR2 alleles and spinal BMD in both men and women. Five of the ten genetic variations reached statistical significance in men, whereas only two reached significance in women (p-value < 0.05). The strongest association in both sexes was found with the allelic variation present in the 5' untranslated region of ESR2 (p-value = 0.004). The alleles may account for up to 2.8% and 0.9% of the spinal BMD variation in healthy men and women, respectively. These results suggest that ESR2 polymorphisms are important in development of bone mass in both men and women.

Mutations of SLC26A4 gene is the second cause of non syndromic deafness. *S. Albert¹, H. Blons², F. Denoyelle¹, D. Feldmann², R. Couderc², E.N. Garabedian¹, C. Petit³, S. Marlin⁴.* 1) Dept of Pediatric ENT, Hosp A.Trousseau, AP-HP, Paris, France; 2) Dept of Biochemistry, Inserm U587, Hosp A.Trousseau, AP-HP, Paris, France; 3) Inserm U587, Institut Pasteur, Paris, France; 4) Dept of Clinical Genetics, Inserm U587, Hosp A.Trousseau, AP-HP, Paris, France.

Sensorineural hearing loss is the most frequent sensorial deficit in child, genetic origin accounting for up to 75% of the cases. It was shown that mutations of the SLC26A4 (PDS) gene were involved either in syndromic deafness characterized by congenital sensorineural hearing loss and goitre (Pendred syndrome), or in congenital isolated deafness (DFNB4). If the prevalence of SLC26A4 mutations in Pendred syndrome is clearly established, it remains to be studied in non syndromic deafness. In this report 101 patients, with non syndromic deafness and inner ear malformations, were genotyped for SLC26A4 using DHPLC molecular screening and sequencing. Seventy eight allelic mutations were observed and thirteen had never been reported. Most mutations were found in exons 6, 10, 16, 19 and 14, some of them had already been described in Pendred syndrome. Prevalence of patients with SLC26A4 mutations was 36.7% and prevalence of compound heterozygous patients was 16%. All patients included in this series had documented deafness (age of onset, severity, type, evolution status), associated with inner ear malformation (enlarged vestibular aqueduct, cochlear dysplasia). None had argument for syndromic disease. Clinical and genotypic data were collected in order to point out correlations. Among patients with PDS mutations, deafness was more severe and more fluctuant than patients with no mutations ($p < 0.05$). In conclusion, SLC26A4 mutations occurred frequently in patients with isolated deafness and inner ear malformation and represents up to 6% of non syndromic deafness cases. In terms of frequency, SLC26A4 is the second gene after GJB2 implicated in non syndromic deafness. Therefore deaf children with inner ear malformation should benefit from SLC26A4 molecular screening.

OPA1 gene mutations are frequently found in sporadic optic atrophies and are also responsible for auditory neuropathy. *P. Amati-Bonneau*¹, *C. Ayuso*², *F. Viala*³, *S. Joriot*⁴, *S. Odent*⁵, *A. Guichel*⁶, *M. Ferré*¹, *C. Hamel*⁷, *P. Belenguer*⁸, *Y. Malthièry*¹, *D. Bonneau*⁶, *P. Reynier*¹. 1) Service de Biochimie et Biologie Moléculaire, CHU Angers, France; 2) Genetic Department, Fundacion Jimenez Diaz, Madrid, Spain; 3) Service de Neurologie, CHU Toulouse, France; 4) Service de Neuropédiatrie, CHU Lille, France; 5) Service de Génétique Médicale, CHU Rennes, France; 6) Service de Génétique Médicale, CHU Angers, France; 7) INSERM U 583 Montpellier, France; 8) Laboratoire de Biologie Cellulaire UMR CNRS 5088, Toulouse, France.

Autosomal dominant optic atrophy (ADOA) (MIM 165500) is characterized by moderate to severe loss of visual acuity with an insidious onset in early childhood, blue-yellow dyschromatopsia and central scotoma. The penetrance of the disease may be as low as 40%. Most cases of ADOA are due to mutations in the OPA1 gene which encodes a dynamine-GTPase involved in the dynamics of the mitochondrial network. A total of 83 OPA1 mutations have been reported so far, and the spectrum of mutations supports the hypothesis that haploinsufficiency is a major mechanism of ADOA. We analyzed 308 unrelated patients affected with optic atrophy. Of these, 40% were familial cases and 60% sporadic. We found 71 (23%) different mutations, 63% and 37% in familial and sporadic cases respectively. A new database of OPA1 gene mutations and SNPs has been set up (<http://lbbma.univ-angers.fr/eOPA1>). We demonstrated that a specific mutation in OPA1 (R455H) was responsible for both optic and auditory neuropathy. The R445H mutation was found in 5 patients from 4 unrelated families affected with optic atrophy and retrocochlear perceptive deafness suggesting a strong genotype-phenotype correlation. This mutation, which affects a conserved amino acid in the GTPase domain of OPA1, was absent in 400 chromosome controls. We studied skin fibroblasts from patients and demonstrated that the R455H mutation leads to the hyperfragmentation of the mitochondrial network. Our results underscore the usefulness of OPA1 testing in sporadic optic atrophies and show that OPA1 mutation may lead to dysfunction in the optic as well as the auditory nerves.

GJB2 gene mutations: genotypic and phenotypic correlation. *D. Feldmann*¹, *H. Blons*¹, *F. Denoyelle*², *E. Garabedian*², *R. Couderc*¹, *C. Petit*³, *S. Marlin*⁴. 1) Dept Biochemistry and Inserm U587, A.Trousseau Hosp, AP-HP, Paris, France; 2) Dept ORL and Cervico-Facial Surgery, Inserm U587, A.Trousseau Hosp, AP-HP, Paris, France; 3) INSERM U587, Institut Pasteur, France; 4) Dept Clinical Genetics and Inserm U587, A.Trousseau Hosp, AP-HP, Paris, France.

GJB2 mutations represent the first cause of congenital non-syndrommic hearing loss in developed countries. We analysed the phenotype and the genotype of 265 patients presenting a homozygote mutation or double heterozygote mutations in GJB2, or the association of a GJB2 mutation with the GJB6 deletion : del(GJB6-D13S1830). Although the 35delG remains the most frequent mutation of GJB2 (68% of the alleles), we identified a total of 30 different mutations of this gene, of which 6 can be considered recurrent: del(GJB6-D13S1830), 310del14, E47X, Q57X, L90P, and V37I. We described 4 new mutations 355del9, 573-574delCA, M151R and Y115X. We have compared the deafness observed in patients homozygous 35delG and in patients with other genotypes. The mutations L90P and V37I were associated with a less severe phenotype than the phenotype of 35delG patients. Intrafamilial variation in the severity of the deafness was observed in 18/50 families (36%) and was not affected by the genotype. The clinical follow-up of the patients showed a hearing level that was stable in 76% (75/99), progressive in 21% (21/99) and fluctuant in 3% (3/99). The evolution of the disease was also independent of the genotype. This result is important for genetic counselling and pathologic studies of deafness caused by GJB2 mutations.

GJB6 and deafness in the Portuguese population. *M. Fialho¹, H. Teixeira¹, T. Matos¹, I. Galhardo², A.R. Santos², A. O'Neill³, S. Almeida⁴, H. Rosa⁵, J.C. Ferreira⁶, O. Dias², M. Andrea², H. Caria⁷, C. Correia¹.* 1) CGBM, Faculty of Science, University of Lisbon, Lisbon, Portugal; 2) Center of ORL Research, Faculty of Medicine, University of Lisbon; 3) ORL Service, Hospital Egas Moniz; 4) Genetics Service, Hospital D. Estefnia; 5) ORL Service, Hospital Garcia de Orta; 6) Obstetrics Service, Hospital Garcia de Orta; 7) Higher School of Health, Polytechnic Institut of Setbal, Setbal, Portugal.

Connexins 26 (Cx26) and 30 (Cx30) are major proteins of gap junctions that play an important role in the process of intercellular communication, and are co-expressed in the cochlea. Half of the cases of nonsyndromic hearing impairment are caused by mutations in the gene encoding the gap junction protein connexin 26 (GJB2). However, 10 to 42 percent individuals, heterozygous for recessive mutations in this gene, are deaf, and no mutation in either the coding region or the splice sites of the other allele has been identified. GJB6, the gene encoding connexin 30, is an obvious candidate, due its structural and expression similarities with GJB2. Some deafness causing mutations were described, namely the T5M point dominant mutation and a 342 kb deletion responsible for recessive hearing loss. This deletion is the second most frequent mutation in the Spanish population and has thus become an important tool in the diagnosis of hereditary prelingual deafness. In the present study we have evaluated 15 Portuguese families with nonsyndromic sensorineural hearing loss in which the affected members have first been screened for GJB2 mutations and shown to have only one mutant allele. Molecular testing then focused on GJB6 gene. Mutation analysis was performed by PCR of the coding region, followed by SSCP. A specific PCR assay to amplify the break-point junction of the 342 kb deletion was used. This report presents the contribution of GJB6 gene for nonsyndromic hearing loss in Portuguese families monoallelic for GJB2 mutations.

Dominant and null alleles of *GJB2* segregating with a *PAX3* mutation in a family with hearing loss. *J.M.*

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Mutations in the *GJB2* gene are the most common genetic abnormality associated with congenital nonsyndromic deafness. While most *GJB2* mutations are recessive, autosomal dominant mutations have also been reported which likely have a dominant negative effect by interfering with the formation of the connexon channels.

We describe a family in which a dominant *GJB2* mutation (R75W) is found in combination with a normal allele or a 35delG mutation. Counseling for this family is complicated by the segregation of a *PAX3* mutation (V29M). The mother of our family has nonsyndromic hearing loss while the father has Waardenburg syndrome. All of their children, three sons, are deaf. One has nonsyndromic hearing loss, and two have Waardenburg syndrome.

The genotypes in our family are complex. The mother is a compound heterozygote for the recessive 35delG(null) and dominant R75W mutations in the *GJB2* gene. One of her sons with Waardenburg syndrome also has both of these mutations, in combination with the *PAX3* V29M mutation. The other son with Waardenburg syndrome has the dominant R75W mutation in the *GJB2* gene and the V29M mutation in *PAX3*. Finally, the son with non-syndromic hearing loss is a 35delG homozygote for the *GJB2* gene.

The complex inheritance of *GJB2* mutations in this family provides an opportunity to characterize the phenotypic effect of a presumed dominant negative mutation (R75W) alone and in combination with a normal connexin 26 protein. The mother described above has isolated hearing loss due to the R75W mutation in combination with a null (35delG) mutation, consistent with the reported inability of the R75W to function properly. Two of the sons have paternally inherited 35delG mutations, yielding individuals who have mutations in two different hearing loss genes (*PAX3* and *GJB2*). This family demonstrates the difficulty in diagnosing and counseling when mutations are segregating in multiple genes.

Genetic basis of hearing loss associated with enlarged vestibular aqueducts (EVA) in Koreans. *S.K. Koo¹, H.J. Park², A.J. Griffith³, S.J. Lee¹, H.S. Jin¹, S.H. Go¹, S.C. Jung¹.* 1) Div Genetic Diseases, NIH, Korea, Seoul; 2) Soree Ear Clinic, Seoul, Korea; 3) National Institute on Deafness and Other Communication Disorders, MD, USA.

Sensorineural hearing loss associated with enlargement of the vestibular aqueduct (EVA) can be associated with mutations of the SLC26A4 gene. In western populations, less than one half of affected individuals with EVA have two mutant SLC26A4 alleles, and EVA is frequently caused by unknown genetic or environmental factors alone or in combination with a single SLC26A4 mutation as part of a complex trait. In this study we ascertained 26 Korean probands with EVA and performed nucleotide sequence analysis to detect SLC26A4 mutations. All subjects had bilateral EVA, and 20 of 26 were sporadic (simplex) cases. Fourteen different mutations were identified, including nine novel mutations. Five mutations were recurrent and accounted for 80% of all mutant alleles, providing a basis for the design and interpretation of cost-efficient mutation detection algorithms. Two mutant alleles were identified in 21 (81%), one mutant allele was detected in 3 (11%), and zero mutant alleles were detected in 2 (8%) of 26 probands. The high proportion of Korean probands with two SLC26A4 mutations may reflect a reduced frequency of other genetic or environmental factors causing EVA in comparison to western populations.

The *USH1C* 216GA splice site mutation results in a 35 base pair mutation. *J.J. Lentz¹, S. Savas¹, S-S. Ng¹, G. Athas¹, P. Deininger², B. Keats¹*. 1) Department of Genetics, Louisiana State University Health Sciences Center, New Orleans, LA; 2) Tulane Cancer Center, Tulane University Health Sciences Center, New Orleans, LA.

Usher syndrome is a heterogeneous group of disorders characterized by both deafness and blindness. The prevalence worldwide is reported to be between 1/16,000 and 1/50,000. Usher can be divided into three sub-types based on clinical severity and progression of sensorineural deafness, retinal degeneration and vestibular dysfunction. Usher type I is characterized by profound hearing loss at birth, vestibular dysfunction and progressive retinitis pigmentosa beginning in early adolescence. Genetic studies have determined the location of 12 genes associated with Usher (*USH1A-G*, *USH2A-C*, and *USH3A, B*). The *USH1C* locus was mapped to chromosome 11p by linkage analysis of Acadian families in Southwestern Louisiana and later shown to be caused by the *USH1C* gene, which encodes a PDZ-domain protein named harmonin. All Acadian patients were homozygous for both a cryptic splice site mutation in exon 3 (216GA), and a 45 base pair VNTR in intron 5 with nine repeats and a base pair change (GT) in the eighth position of the eighth repeat (9VNTR(t,t)). The finding of complete linkage disequilibrium between these events raised the possibility that homozygosity for both changes may be required for expression of the phenotype. Bitner-Glindzicz et al. (Nat. Genet. 26:56-60, 2000) reported that the 216GA mutation creates an in-frame deletion of 39 base pairs and results in an unstable transcript. In order to analyze the cause of this instability, we transfected HeLa cells with two constructs, one containing 216G and the other 216A. RT-PCR analysis demonstrated that the mRNA size difference is 35 bases not 39 bases, and this result was confirmed in lymphoblast cell lines from patients. A size difference of 35 base pairs means that the 216GA mutation results in a frame-shift and the transcript would encode a highly truncated, non-functional protein. Thus, the instability of the *USH1C* mRNA is explained by the out-of-frame cryptic splice site mutation in exon 3.

Otoferline : The trap of the neonatal hearing loss screening with evoked otoacoustic emissions. *A. Marcolla¹, H. Blons², D. Feldmann², F. Denoyelle¹, E.N. Garabedian¹, R. Couderc¹, C. Petit³, S. Marlin⁴.* 1) Dept of Pediatric ENT, Hospital A.Trousseau, AP-HP, Paris, France; 2) Dept of Biochemistry, Inserm U587, Hospital A.Trousseau, AP-HP, Paris, France; 3) Inserm U587, Institut Pasteur, Paris, France; 4) Dept of Clinical Genetics, Inserm U587, Hospital A.Trousseau, AP-HP, Paris, France.

Since 1994, more than 70 loci for non syndromic hearing loss NSHL have been identified and 35 different genes cloned. One of them, GJB2, encoding connexin 26, is involved in the most frequent form of NSHL: DFNB1. But the prevalence of the other genes in NSHL is unknown. The OTOF gene encodes otoferlin, a protein expressed in the inner hair cells and probably involved in the fusion of the synaptic vesicles in the cochlear neuro-epithelium. Mutations in OTOF are responsible for a congenital profound non syndromic deafness : DFNB9. The characteristic of DFNB9 is the transient persistence of evoked otoacoustic emissions (OAE), leading to either a false negative results in hearing loss screening programmes using this technique or to a diagnosis of auditory neuropathy. In two families with a DFNB9 phenotype, we have screened the OTOF coding sequence and identified two new mutations IVS44+1G->A and P490R associated in trans with Q829X, a frequent mutation in the Spanish population. In addition, Q829X was studied in 120 families showing prelingual, bilateral without inner ear malformation and severe or profound NSHL compatible with a recessive transmission and without GJB2 mutation. One homozygous patient was identified. In this patient OAE performed at 10 years of age were negative. Indeed, OAE usually disappear after one year of age. In conclusion, the OTOF gene was involved in 3/123 patients (2.5%) and should be screened in subjects with profound hearing impairment.

Screening of the *GJB2* (*Connexin 26*) Promoter Region Identifies a 10 Base Pair Deletion in Children with Bilateral Sensorineural Hearing Loss. *J. McCallum*¹, *C. Stolle*², *D. Yaeger*¹, *I.D. Krantz*¹. 1) Division of Human Genetics; 2) Division of Clinical Labs, The Children's Hospital of Philadelphia, PA.

Mutations in *GJB2* are the most common genetic cause of congenital bilateral non-syndromic recessive sensorineural hearing loss (BLSNHL). Individuals with BLSNHL and heterozygous mutations in *GJB2* have been consistently reported at higher frequencies than in the general population. It has been postulated that the second mutation necessary for BLSNHL in these individuals may be in other genes, or in regions of *GJB2* that are not routinely screened. In support of this, BLSNHL in some *GJB2* heterozygotes has been explained by a trans deletion of *GJB6* (*Connexin 30*). We hypothesized that, in individuals with BLSNHL and a single *GJB2* mutation, the second mutation may be in regulatory regions not presently screened. We have screened the promoter region of *GJB2* in 84 children with BLSNHL (8 *GJB2* homozygotes, 8 *GJB2* heterozygotes, and 68 with no identifiable *GJB2* mutations). A 10 nucleotide deletion (-3866 del 10) in the promoter encompassing part of a highly conserved consensus sequence for an MGF binding site was identified in 8 children, 6 of whom are *GJB2* mutation negative and 2 of whom are 35delG/M34T compound heterozygotes. The M34T change is now felt to represent a polymorphism. The -3866 del 10 was also seen heterozygously in 5/100 control samples. This indicates that heterozygous -3866 del 10 is not sufficient to cause BLSNHL. Protein binding to the MGF consensus sequence of *GJB2* in mammary cells up-regulates *GJB2* expression during lactation. Disruption of this site would likely down-regulate expression. If a similar mechanism occurs in the cochlea, the -3866 del 10 may account for some of the unfound changes in children with BLSNHL and no identifiable *GJB2* mutations or in trans with those with a heterozygous mutation. Alternately, the -3866 del 10 may itself be a polymorphism or might have a mild modifier effect on the phenotype of children with two pre-existing mutations. To date no homozygotes for this deletion have been identified. Studies are underway to determine the effect of this promoter deletion on *GJB2* gene expression and its role in the pathogenesis of BLSNHL.

Significance of the M34T variant of the GJB2 gene as a cause for deafness. *A. Pandya*¹, *K. Withrow*¹, *X-J. Xia*¹, *K.S. Arnos*², *V. Norris*², *W.E. Nance*¹. 1) Dept Human Genetics, Virginia Commonwealth University, Richmond, VA; 2) Dept. of Biology, Gallaudet University, Washington DC.

Mutations in the gap junction beta 2(GJB2) gene are the cause for more than 50% of profound pre-lingual genetic deafness. Although one specific mutation, 35delG accounts for 60%-70% of all pathogenic sequence variants, more than 80 mutations have been reported. Amongst these, the pathogenic role of the M34T substitution in GJB2 has been quite controversial. Early case reports and functional data in the xenopus oocyte system suggested that M34T might be a functionally significant dominant allele. Others have suggested that M34T may contribute to recessive hearing loss (HL). However, the presence of hearing compound heterozygotes with M34T and a known pathogenic GJB2 mutation, and similar frequencies of M34T in deaf and hearing control populations have raised the question of whether this change may represent a normal variant. To further evaluate the role of M34T, we have examined data from our national DNA repository from deaf individuals. In our repository, GJB2 alterations were found in 419 (37.2%) of 1125 deaf probands. The M34T allele was identified in 21(1.8%) deaf probands, including 18 heterozygotes, 1 homozygote, and 2 compound heterozygotes. Of these, 15 were from multiplex & 6 from simplex families. In 19 families with only M34T mutation, 42 family members were either known or obligate M34T carriers. In relatives who were not probands, 6 M34T carriers were deaf and 17 were hearing. If M34T is a dominant allele, our data suggest that it has a penetrance of 6/23 (26.1%). One 35delG/M34T compound heterozygote had unilateral HL as did his M34T heterozygote father and his paternal grandfather. The other compound heterozygote carried a H100Y allele in addition to M34T. The M34T homozygote was also deaf, although his HL was attributed to childhood meningitis. In all, 21 of 1125(1.87%) deaf or hard of hearing probands while 4 of 173 hearing subjects (2.3%) in the repository carried at least one M34T mutation. We conclude that the M34T allele is unlikely to be a cause of deafness, although the possibility that it may represent a hypomorph with low penetrance cannot be excluded.

SCAIP to identify Usher type 1 gene mutations in French patients: a pilot study. *A.F ROUX, V. FAUGERE, N. PALLARES-RUIZ, A. VIELLE, S. CHAMBERT, M. CLAUSTRES.* Genetics, CHU Montpellier, Montpellier, France.

Usher syndrome is the most common recessive disorder that associates hearing loss (HL) and retinitis pigmentosa (RP). It is both clinically and genetically heterogeneous. Current classification divides Usher syndrome in three distinct clinical types. Usher syndrome type 1 (USH1) is the most severe form characterized by profound congenital hearing loss, vestibular dysfunction and prepubertal onset of retinitis pigmentosa. To date seven loci, USH1A-G, have been mapped. Of them, 5 responsible Usher genes have been cloned: MYO7A, CDH23, PCDH15, USH1C and SANS. Most of MYO7A mutations are rare or private and mutation analysis requires the entire gene scanning. Therefore, we had initially chosen DHPLC to study the MYO7A gene. However, we encountered a high proportion of non pathogenic variants in our population. Moreover, too many domains of denaturation could be observed within one exon preventing thus any optimization of the analyses. Finally, we have applied the Single Condition Amplification/Internal primer method (SCAIP), initially developed for the dystrophin gene (Flanigan et al. 2003) to MYO7A and extended this performing approach to the CDH23 gene. One hundred samples are being currently analyzed: the screen already reveals recurrent mutations in our population for both genes (R1240Q and 223delG for MYO7A, IVS45-9G/A in CDH23) as well as new pathogenic mutations (IVS41 +1G/A ; A198T ; R1873W ; G163R in MYO7A). Our results also raise questions about the pathogenic status of some known mutations (T1566M and Y1719C). Because the whole coding region and intronic borders are analyzed, complex haplotypes are identified. These data will be integrated for genotype/phenotype correlations. This study shows that molecular diagnosis is becoming accessible to French patients and that prenatal diagnosis can be offered with an adequate genetic counseling.

Screening for mutations in alpha connexins in patients with hearing impairment that are negative for beta connexin genes. *M. Ventayol¹, E. Ballana¹, R. de Cid¹, P. Gasparini², X. Estivill^{1,3}*. 1) Genes and Disease, Center for Genomic Regulation, Barcelona, Spain; 2) Telethon Institute of Genetic and Medicine, Naples, Italy; 3) Experimental and Health Sciences Department,UPF, Barcelona, Spain.

Non-syndromic hearing impairment has a high incidence in general population affecting about 1 on 1000 newborns in developed countries, with genetic causes accounting for more than 60% of the cases. More than 80 loci and 30 genes have been related to non-syndromic hearing loss. Nevertheless mutations in a single beta-connexin gene (GJB2) are responsible for up to 50% of cases with an autosomal recessive pattern of inheritance, which is the most common one. Connexins are transmembrane proteins, the forming units of gap junctions that are basic for the maintenance of homeostasis in the cochlea. Twenty human connexin genes have been identified, they are subdivided in three groups: alpha, beta and gamma. Three beta connexins genes (GJB2, GJB6 and GJB3) and one alpha connexin (GJA1) have been associated with deafness, so far. Alpha connexins represent half of all connexins identified until now, therefore they should not be excluded as genes involved in hearing impairment. Gja4 is expressed in the mouse cochlea, being therefore a good candidate gene for deafness. We have performed a mutational screening in the alpha connexin 37 gene (GJA4) in 150 non related individuals belonging to Spanish families in which neither a mutation in the GJB2 gene nor the A1555G mitochondrial mutation have been identified. Denaturing high performance liquid chromatography methodology (dHPLC) has been used for the screening of mutations in GJA4 gene, where four single nucleotide substitutions have been identified so far. Two of these changes (132g>a, 147a>g) correspond to SNPs already described in public databases, while the other two (-8c>t, -37c>t) localise to the 5' UTR and have not yet been reported but they appear at very low frequencies (one individual for each change). Further studies should be performed in order to shed light on the potential role of this changes regarding the deafness phenotype. Other alpha connexin genes such as GJA1 or GJA10 are also under study to elucidate their role on hearing impairment.

A deafness associated Connexin allele that results in decreased expression. *E. Wilch*¹, *M. Zhu*², *S. Lebeis*⁶, *M. Regier*³, *J. Elfenbein*⁴, *R. Fisher*⁵, *K. Friderici*^{1,2,5}. 1) Genetics Program; 2) Department of Microbiology and Molecular Genetics; 3) Department of Epidemiology; 4) Department of Audiology and Speech Sciences; 5) Department of Pediatrics and Human Development, Michigan State University, East Lansing, MI; 6) Graduate Program in Microbiology and Molecular Genetics, Emory University, Atlanta, GA.

Mutations in GJB2, the gene coding for the gap junction protein Connexin 26 (Cx26), may account for 50% of cases of recessively inherited, nonsyndromic hearing loss (NSHL). Despite a current reported total of ~70 mutations of Cx26 causing recessive NSHL, screening efforts consistently reveal a significant number of deaf individuals bearing only one identified mutant Cx26 allele. In non-Jewish Caucasian populations, this mutation is most commonly 35delG, which is present at a carrier frequency of about 3% in these populations.

In our studies of a large extended Michigan family of northern European descent we identified several deaf individuals who are homozygous 35delG/35delG. However, four other deaf family members are 35delG heterozygotes; all share a common haplotype in the region containing the GJB2 gene on their non-35delG chromosome. Sequencing of the coding regions and splice sites of GJB2 and GJB6 did not reveal any potential additional mutation in the 35delG heterozygous proband. Heterozygosity of our proband at various markers within and around GJB2 indicates that this individual is not deleted for either GJB2 or the 342kb interval upstream of GJB2 and including the 5end of GJB6 that segregates with deafness when present in *trans* with 35delG. We developed a PCR-based, cDNA-specific assay to determine whether Cx26 was transcribed from the non-35delG chromosome. Buccal cells from the proband produce mRNA only from the 35delG chromosome.

This supports our hypothesis that an unidentified regulatory mutation of GJB2, when present in *trans* with 35delG, is responsible for hearing loss in these subjects. None of the previously reported mutations implicated in recessive Cx26 hearing loss affect regulation of Cx26 expression.

Mutations in the *VPS33B* gene, encoding a regulator of SNARE-dependant membrane fusion, cause ARC syndrome. *P. Gissen*^{1,2}, *C.A. Johnson*¹, *N.V. Morgan*¹, *E. Genin*³, *P.J. McKiernan*², *D.A. Kelly*², *E.R. Maher*². 1) Section of Medical and Molecular Genetics, University of Birmingham, Birmingham, UK; 2) The Liver Unit, Birmingham Childrens Hospital, Birmingham, UK; 3) INSERM U535, France.

Introduction: ARC syndrome (OMIM 208085) is an autosomal recessive disorder characterised by Arthrogryposis multiplex congenita, Renal dysfunction and neonatal Cholestasis with bile duct hypoplasia and low gamma glutamyl transpeptidase (GGT) activity. Platelet dysfunction is common. Affected infants fail to thrive and usually die in the first year of life. Methods and Results: We used autozygosity mapping to map the *ARC* locus to 15q26.1. We then screened the candidate genes in the region by direct sequencing and identified 9 germline mutations in the *VPS33B* gene in 14 ARC kindreds (Nat. Genet. 36 400-405 (2004)). One mutation was present in 8 unrelated Pakistani families, who shared haplotype around the gene. Haplotype analysis allowed us to calculate the age of this founder mutation to approximately 900-1000 years. *VPS33B* encodes a homologue of the class C yeast vacuolar protein sorting *Vps33* gene that contains a Sec1-like domain important in the regulation of vesicle to target SNARE complex formation. Immunofluorescence showed that overexpression of *VPS33B* caused perinuclear clustering of late endosomes, identifying a role in mobilising these membrane-bound organelles. Immunostaining suggested disordered trafficking of the polarised integral membrane proteins. Summary and Conclusions: ARC is the first human disorder associated with mutations in a gene involved in regulation of the SNARE-mediated mechanism of membrane tether and fusion. Studies to elucidate the function of the *VPS33B* gene product are in progress. *VPS33B*.

The mutational spectrum of *NSDHL* in CHILD syndrome: Functional implications. K. Grzeschik¹, L. Leveleki¹, A. König², D. Bornholdt¹, P. Rölle¹, R. Happle². 1) Zentrum für Humangenetik, Philipps-Universität, Marburg, Deutschland; 2) Klinik für Dermatologie und Allergologie, Philipps-Universität, Marburg, Deutschland.

CHILD syndrome (Congenital Hemidysplasia with Ichthyosiform Nevus and Limb Defects, MIM 308050), is an X-linked dominant, male-lethal trait characterized by an inflammatory nevus that usually shows striking lateralization with strict midline demarcation as well as ipsilateral hypoplasia of the body. Recently, we were able to demonstrate that this trait is caused by mutations in the gene *NSDHL* (NAD(P)H steroid dehydrogenase-like protein) encoding a 3-hydroxy-steroid dehydrogenase functioning in the cholesterol biosynthetic pathway. *NSDHL* maps to Xq28.

We report the results of mutational analysis in 30 familial and sporadic cases. The phenotype appears to be caused by loss of function because it can be associated with nonsense- and missense mutations as well as with deletions eliminating several exons or the complete gene.

Amino acids of *NSDHL* located outside the predicted functional domains (co-factor binding site, catalytically active site, transmembrane helix) which are highly conserved in evolution may pinpoint positions of potential functional importance. We generated by mutagenesis human *NSDHL* transgenes reflecting missense-mutations observed in CHILD patients or having altered other potentially functionally important sites. These constructs are transiently expressed in fibroblasts of a male CHILD patient containing either the mutated or the intact X-chromosome. The subcellular distribution of the protein is monitored. Complementation analysis by transfer of mutated human *NSDHL* into the *erg26^{ts}* yeast strain, which is mutated in the orthologous gene, *ERG26*, suggests functional differences between mutants, which are not reflected in the human phenotype.

***UNC13D* and *PRF1* mutations in childhood patients with hemophagocytic lymphohistiocytosis.** H.C. Hennies¹, K. Beutel², S. Schmidt¹, F. Oyen², S. Diler³, H. Kabisch², P. Nürnberg¹, R. Schneppenheim², G. Janka², U. zur Stadt². 1) Molecular Genetics and Gene Mapping Center, Max-Delbrück Center, Berlin, Germany; 2) Pediatric Hematology and Oncology, Childrens University Hospital, Hamburg, Germany; 3) Medical Biology and Bone Marrow Bank, University of Istanbul, Turkey.

Familial hemophagocytic lymphohistiocytosis (FHL) is an autosomal recessive disease affecting young children. It presents as a severe hyperinflammatory syndrome with activated macrophages and T lymphocytes. Mutations in the perforin 1 gene (*PRF1*) were reported in FHL-2 in 15-50% of all cases. Defective granule exocytosis caused by mutations in *UNC13D* has recently been described as the defect underlying FHL-3. Both types are phenotypically indistinguishable. We analyzed a large cohort of 61 patients with hemophagocytic lymphohistiocytosis from different geographic origins by linkage analysis and direct sequencing. We identified mutations in 28 samples investigated, 18 in *PRF1* (30%) and 10 in *UNC13D* (16%). Besides several known mutations, novel deletions, missense and nonsense mutations were detected in both genes throughout the coding regions. In 24 patients from Turkey, 13 had mutations in *PRF1* (54%) and 4 had mutations in *UNC13D* (17%). The mutation Trp374X, found in 11/13 patients with *PRF1* mutation from Turkey, was the only mutation observed repeatedly in patients from a common geographic origin. Surprisingly, only 7/28 patients from Germany showed mutations in either *PRF1* (3; 11%) or *UNC13D* (4; 14%). Furthermore, 4 out of 9 patients from other countries showed mutations in one of these genes. We detected only one mutation in *UNC13D* repeatedly in 10 heterozygous patients so far. Moreover, a genome-wide scan for a novel locus for FHL is under way using homozygosity mapping in a large consanguineous family from Turkey. Our results indicate that FHL-2 and FHL-3 account for more than 70% of FHL cases from Turkish origin, however, mutations were identified in only 25% of cases from German descent. Our data demonstrate extensive genetic heterogeneity in FHL, differences in epidemiology depending on the ethnicity, and the importance of further, yet unknown loci for the etiology of FHL.

Smith-Lemli-Opitz syndrome: Carrier frequency and spectrum of *DHCR7* mutations in Poland. *M. Krajewska-Walasek¹, E. Ciara¹, E. Malunowicz¹, B. Goryluk-Kozakiewicz¹, M. Witsch-Baumgartner², A. Jezela-Stanek¹, M. Piotrowicz³, K. Chrzanowska¹, J.S. Wayer⁴, M.J.M. Nowaczyk⁴, E. Popowska¹, G. Utermann².* 1) Dept. of Genetics, Childrens Memorial Health Institute, Warsaw, Poland; 2) Institute of Medical Biology and Human Genetics, Innsbruck, Austria; 3) Polish Mothers Hospital Research Institute, Lodz, Poland; 4) Dept. Pathology and Molecular Medicine, Faculty of Health Sciences, McMaster University, Hamilton, Canada.

SLOS is an autosomal recessive disorder of cholesterol biosynthesis caused by mutations in *DHCR7* gene. To date, most studies of SLOS carrier rates have dealt with Caucasians of Western European origins and were based on the observed carrier rates for the most common mutation in those populations, IVS8-1G>C. In Central Europe the most common mutations are W151X and V326L.

We performed a molecular analysis of the *DHCR7* gene 37 SLOS ethnic Polish patients. We identified 15 different *DHCR7* mutations. W151X (30% of alleles) and V326L (27.9%) accounted for 59% of all *DHCR7* mutations in Polish patients. Five missense mutations, I178N, M270V, G309S, L360P, R443H have only been observed in Polish patients. Using a previously described PCR-RFLP assay we screened 2900 anonymous blood samples from random newborn screening blood spot cards for the presence of the W151W (1650 blood spots) and V326L (1250) mutations. Thirty W151X carriers and 9 V326L carriers were identified. Given that these two mutations constitute 59% of the mutations observed in our population, the overall heterozygote carrier frequency may be as high as 3.32% to 5.22%, and the incidence of SLOS in Poland as high as 2.76 4.6 in 10,000. The discrepancy between calculated and observed incidence could be due to undiagnosed mild cases, misdiagnosed severe cases, or fetal loss of W151X homozygotes. In our series only one W151X/W151X infant was identified. More comprehensive incidence studies, including prenatal surveillance, are needed to determine if SLOS is as common as predicted by the very high 3-5% carrier frequency determined in this study.

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2000 animal Bovine Research pedigree development and use; potential for human relevant gene discovery. *R.G. Snell¹, S. Berry¹, S. Davis¹, J. Pearson¹, P. Oxley¹, A. Stanfield¹, C. Ford¹, G. Stanley², S. Harcourt², J. Hooper², R. Spelman².* 1) Bovine Genetics, ViaLactia Biosciences, Auckland, New Zealand; 2) Livestock Improvement Corporation, Private Bag 3016, Hamilton, NZ.

The New Zealand dairy industry has a well developed molecular genetic program to identify markers and genes associated with both positive and negative traits. As part of our strategy we have generated a reciprocal cross of Holstein-Friesian and Jersey breeds to produce six F1 bulls and subsequently 850 F2 females. The complete pedigree has been genotyped with 200+ microsatellite markers for QTL and gene identification. Extensive phenotype recording of the F2 animals is in progress including milk production and milk composition. All the F2 animals have undergone three endocrine challenges, having been administered glucose, epinephrine and TRH. Growth rate, disease status and a large range of other characteristics have also been recorded. The soon to be released draft of the bovine genome will make the co-localisation of bovine and human traits simple. Although some genes will be discovered using this pedigree as a consequence of the dairy industries interests the potential to measure other characteristics which are possibly more relevant to human, exists. We are very interested in working with other researchers to take full advantage of this unique resource. The trial design and list of traits measured or currently being measured and results to date will be presented.

Mutations in human TBX4 cause lower limb malformations in small patella syndrome. *E. Bongers¹, P. Duijf¹, S. van Beersum¹, J. Schoots¹, A. van Kampen², A. Burckhardt³, B. Hamel¹, F. Loan⁴, L. Hoefsloot¹, H. Yntema¹, H. van Bokhoven¹, N. Knoers¹.* 1) Dept Human Genetics, Univ Medical Ctr, Nijmegen, Netherlands; 2) Dept Orthopaedic Surgery, Univ Medical Ctr Nijmegen, Netherlands; 3) Dept Orthopaedic Surgery, Kantonsspital Olten, Switzerland; 4) Dept Medical Genetics, Genetika Plzn, Czech Republic.

Small patella syndrome (SPS; MIM 147891) is an autosomal dominant skeletal dysplasia characterized by patellar aplasia or hypoplasia and pelvic anomalies, including disrupted ossification of the ischia and inferior pubic rami. In addition, femur and foot anomalies may accompany SPS. We identified an SPS candidate region of 5.6-cM on chromosome 17q22 by haplotype analysis. Inspection of genes in the SPS critical region revealed the TBX4 gene as a positional candidate. TBX4 belongs to the T-box gene family, encoding transcription factors characterized by a strongly conserved DNA-binding motif. Mutations in several T-box genes are associated with human developmental disorders, including TBX3 mutations in ulnar-mammary syndrome and TBX5 mutations in Holt-Oram syndrome, both characterized by severe malformations of the upper limb. Studies in model animals have indicated an important role for TBX4 in lower limb development. In chickens, misexpression of dominant-negative Tbx4 results in legless phenotypes and in deformed and hypoplastic pelvis. We found putative loss-of-function mutations in TBX4 in 6 families with SPS, including one nonsense, two missense-, one frameshift- and one splice-site mutation, and skipping of exon 7. Haploinsufficiency appears to be responsible for dominantly inherited SPS in at least some of the families. The phenotype associated with heterozygous TBX4 mutations suggest that this gene is involved in late stages of skeletal development. The present identification of TBX4 mutations in SPS patients emphasize the crucial role of TBX genes limb specification: TBX3 and TBX5 in upper limb development and TBX4 in development of the lower limb.

Screening for novel mutations in conserved intronic sequences of the CFTR gene in patients with cystic fibrosis.

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Cystic fibrosis (CF) is a recessive disease affecting 1 in 2500 new born in the Caucasian population. It is characterized by obstructive pulmonary disease, pancreatic insufficiency and congenital bilateral absence of vas deferens (CBAVD) in males. CF is caused by over 1200 different mutations in the CFTR gene. The majority (83%) of such mutations are found in the coding region of the gene. However, a considerable number of CF patients have unknown mutations even after exhaustive sequencing of the entire coding sequence. The number of these unidentified cases is higher in patients presenting with milder forms of the disease such as pancreatic sufficiency (PS). We suspect that there are yet to be identified mutations in sites of intronic regulatory elements within the CFTR locus which currently fall outside the scope of standard CF mutation screening. We have identified conserved non-coding sequences by comparative DNA sequence alignment between human and other species to help pinpoint new genomic sites within the CFTR locus with putative functional significance. We scanned the 220 kb CFTR region and selected more than 40 segments of DNA, each larger than 50 bp in size, with high sequence conservation in a number of species including fugu. These conserved non-coding DNA segments might play roles in the regulation of proper expression of the CFTR gene. We performed mutation screening by DNA sequencing initially targeting 10 selected sites on 93 patient DNA samples (33 CBAVD, 27 PS, 33 PI patients) from whom there were only one or no mutations identified in the CFTR gene. So far, we have identified three different single-nucleotide alterations, each found in unrelated CBAVD patients with the following CFTR genotype: IVS8-5T/unknown, F508/unknown and unknown/unknown. We are currently mutation screening additional candidate regions and increasing our study population to include CF families with incomplete CFTR genotype as well as control samples consisting obligatory carriers and the general population.

Gene-Oriented File System: The Development of A Hermansky-Pudlak Syndrome Database (HPSD). *W. Li^{1, 3}, P. Liang², R.T. Swank¹.* 1) Department of Molecular & Cellular Biology, Roswell Park Cancer Institute; 2) Department of Cancer Genetics, Roswell Park Cancer Institute, Buffalo, NY 14263, USA; 3) Institute of Genetics & Developmental Biology, Chinese Academy of Sciences, Beijing, China.

Hermansky-Pudlak Syndrome (HPS, OMIM 203300) is a genetically heterogeneous disorder characterized by oculocutaneous albinism, prolonged bleeding and pulmonary fibrosis due to abnormal vesicle trafficking to lysosomes and related organelles such as melanosomes and platelet dense granules. This HPS database (**HPSD**) is to provide composite, annotative, and curative data that is distributed in a variety of public databases or predicted by bioinformatics servers for the recently cloned human and mouse HPS genes, as well as the genes responsible for HPS related syndromes, such as Chediak-Higashi syndrome (CHS), Griscelli syndrome (GS), Oculocutaneous albinism (OCA), and Ocular albinism (OA). This HPSD is designed by using a unique **gene-oriented file (GOF)** format. Seven blocks (genomic, transcript, protein, function, mutation, phenotype, and reference) are carefully curated in each user-friendly GOF entry. The HPSD emphasizes pair-wised human and mouse GOF entries. The genes included in this database (currently 56 in total) are arbitrarily divided into three categories: **(1) Human HPS, (2) Mouse HPS, (3) HPS Related Syndromes**. We believe that these very informative and peer-reviewed GOF files will be a shortcut to utilize the web-based information for the emerging interdisciplinary studies of HPS in *Genetics, Cell Biology, Biochemistry, Immunology, Neurology, and Medicine*. To access the HPSD, use this URL: <http://falcon.roswellpark.org/HPSD.htm/>.

A Novel Loss of Function Mutation in the *MSX2* Homeobox Gene in a Family with Foramina Parietalia

Permagna (PFM). V. Bernier¹, M. Ghassibé², L.M. Boon^{2, 3}, M. Vikkula². 1) Dept of Pediatrics, CHC, Liège, Belgium; 2) Lab of Human Mol Genetics, Christian de Duve Inst & Université catholique de Louvain, Brussels, Belgium; 3) Center of Vascular Anomalies, Université catholique de Louvain, Brussels, Belgium.

Parietal foramen is a normal osseous channel coursing through the three tables in the superior parietal region of the skull. Enlarged parietal foramina (PFM, FPP, MIM #168500) are secondary to an ossification default of the parietal fontanelles. They are bilateral and can be up to 2 cm or more in diameter. They may be familial, with an autosomal dominant inheritance. Surgical intervention is sometimes required. PFM can be caused by haploinsufficiency of the *ALX4* gene on 11p11.2, being part of the DEFECT 11 syndrome (MIM #601224), or as an isolated feature, due to loss of function and missense mutations in the *MSX2* gene on 5q34-q35. Altogether, 6 families with such mutations have been reported. *MSX2* belongs to the *MSX* homeobox gene family and codes for a transcription factor. *Msx2*-deficient mice show defects of the skull ossification and persistent calvarial foramen. In contrast, a gain of function mutation in *MSX2* was shown to cause autosomal dominant craniosynostosis type 2 (CRS2, #MIM 604757), also known as craniosynostosis Boston-type (CSB). CRS2 is characterized by the premature fusion of calvarial sutures leading to abnormal skull shape. Our aim is to characterize the PFM causing mutation in a family with 7 members showing persistent cranial foramina without associated symptoms except for a venous malformation in the foramen in one individual. A novel mutation was detected in all the affected members, a duplication of 17 nucleotides leading to a change in the open reading frame followed by a premature stop codon. This duplication is predicted to cause haploinsufficiency due to the abolition of the DNA binding domain. Moreover, two previously reported substitutions cause reduced *MSX2* affinity for DNA and thus lead to the formation of PFM due to haploinsufficiency. These results confirm the dosage effect of *MSX2* during development. (<http://www.icp.ucl.ac.be>) (vikkula@bchm.ucl.ac.be).

DTDST mutations in Finland and in the rest of the world: an unfolding story. *L. Bonafe*¹, *J. Hästbacka*², *A. Rossi*³, *A. de la Chapelle*⁴, *A. Superti-Furga*¹. 1) Div Molecular Pediatrics, Univ Lausanne, Lausanne, Switzerland; 2) Dept Medical Genetics and Hospital for Children and Adolescents, University of Helsinki, Finland; 3) Dept of Biochemistry, University of Pavia, Italy; 4) Human Cancer Genetics Program, The Ohio State University, Columbus, Ohio.

A small proportion of individuals with Diastrophic Dysplasia (DTD) in Finland are not homozygous for the common Finnish mutation IVS1+2TC. In 7 of these individuals we found a hitherto undescribed mutation, T512K, that was compounded with the Finnish mutation. This rare mutation was not found in 204 Finnish and in 150 non-Finnish controls. We then analyzed the Finnish family reported in 1970 as de la Chapelle dysplasia using blood DNA from parents and an unaffected sister and paraffin block-extracted DNA from the two affected stillborns. This family was found to segregate two mutations, T512K and R492W. The affected individuals were homozygous for T512K, father was heterozygous for T512K, mother and sister were compound heterozygous for T512K and R492W (*trans* phase confirmed by PCR cloning). This proved that T512K is pathogenic but, surprisingly, R492W is not in spite of its position in a transmembrane domain. We then re-screened our collection of families with DTDST dysplasias. T512K was not found in over 100 non-Finnish pedigrees with DTDST dysplasias. Conversely, R492W was found in 7 Finnish and 5 non-Finnish controls, and reanalysis of 3 DTD pedigrees in which R492W had been identified revealed the presence of still other, previously unidentified mutations that had gone undetected. We conclude that (1) T512K is a second, rare pathogenic mutation apparently restricted to the Finnish population; (2) T512K is seen mostly compounded with IVS1+2TC because of its higher frequency, the family reported here being the only example of homozygosity; (3) de la Chapelle dysplasia (MIM 256050) is indeed part of the DTDST dysplasia spectrum and corresponds to atelosteogenesis 2; (4) there is vast mutation heterogeneity in DTDST with 57 different pathogenic mutations identified today; in non-Finnish populations, 5 recurrent mutations account for 65% of pathogenic alleles.

No Mutation in the *VG5Q* Gene in Six Patients with Klippel-Trenaunay Syndrome. *L.M. Boon*^{1, 2}, *V. Wouters*², *A. Domp Martin*³, *E. Kajantie*⁴, *I. Kaitila*⁴, *F. Forzano*⁵, *E. Thompson*⁶, *M. Vikkula*². 1) Center for Vascular Anomalies, Clin Univ St-Luc, Université catholique de Louvain, Brussels, Belgium; 2) Lab of Human Molecular Genetics, Christian de Duve Institute & Université catholique de Louvain, Brussels, Belgium; 3) C.H.U-Dept of Dermatology, Caen, France; 4) Hospital for Children and Adolescents, Faculty of Medicine, University of Helsinki, Finland; 5) Clinical Genetic Unit, Galliera Hospital, Geneva, Italy; 6) Laboratory of Genetics, Womens and Childrens Hospital, North Adelaide, Australia.

Klippel-Trenaunay syndrome (KTS) is a rare congenital malformation characterized by cutaneous capillary malformations (port-wine stain), soft tissue and bony hypertrophy, and venous and lymphatic malformations. Alvoet *et al.* (1992) showed evidence that KTS occasionally shows familial aggregation. In addition, they found isolated vascular lesions to be overrepresented in relatives of KTS patients. The cause of the disorder is unknown. Whelan *et al.* (1995) reported a girl with symptoms reminiscent of KTS associated with reciprocal translocation (5;11)(q13.3;p15.1). This suggested that the disorder in this patient may be due to a single gene defect. Recently, in 5 individuals, Tian *et al.* (2004) identified an amino acid substitution (E133K), not present in 200 controls, in a gene they called *VG5Q* located in 5q13.3. The clinical characteristics of these individuals were not described, raising the question about the phenotype linked to the mutation. It is shown that *VG5Q* protein acts as a potent angiogenic factor in promoting angiogenesis, and suppression of *VG5Q* expression inhibits vessel formation. *In vitro*, *VG5Q* is expressed in blood vessels and is secreted as vessel formation initiates. On the basis of these results, we screened the *VG5Q* gene from blood DNA of six KTS patients with classical clinical characteristics, by SSCP (Single Strand Conformation Polymorphism) and HD (Heteroduplex) techniques and sequencing analysis. The results of the screening showed no mutation in the *VG5Q* gene. This suggests that the *VG5Q* gene is not the only gene involved in KTS and other causative genes should be implied. (vikkula@bchm.ucl.ac.be) (<http://www.icp.ucl.ac.be/vikkula>).

Involvement of *IRF6* in Van Der Woude Syndrome (VWS), Popliteal Pterygium Syndrome (PPS) and Isolated Cleft Lip and Palate (CL/P). M. Ghassibe¹, N. Revencu^{1, 2}, B. Bayet³, K. Devriendt⁴, L. Van Maldergem⁵, D. Genevive⁶, Ph. Pellerin⁷, O. Boute⁸, S. Manouvrier-Hanu⁸, R. Vanwijck³, Y. Gillerot^{2, 5}, C. Verellen-Dumoulin², M. Vikkula¹. 1) Lab of Human Mol Genetics, Christian de Duve Inst & Univ catholique de Louvain, Brussels, Belgium; 2) Center for Human Genetics, Clin Univ St-Luc & Univ catholique de Louvain, Brussels, Belgium; 3) Centre Labiopalatin, Clin Univ St-Luc, Brussels, Belgium; 4) Center for Human Genetics, Leuven, Belgium; 5) Centre de Génétique Humaine, Gerpennes, Belgium; 6) Génétique Médicale, Hôpital Necker-Enfants Malades, Paris, France; 7) Chirurgie plastique, Hôpital Roger Salengro, CHU, Lille, France; 8) Génétique Clinique, Hôpital Jeanne de Flandre, Lille, France.

Normal development of lips and palate is a dynamic, highly regulated, and complex process. From mouse models, human syndromes, association and expression studies, a wide range of proteins, such as transcription factors, growth factors and signaling molecules are shown to be involved in this process. When the structure or expression of one or more of these genes is modified, a cleft of the lip only (CL), palate only (CP), or lip and palate (CLP) may occur. Despite recent investigations on the mode of inheritance and interaction of different factors, genetics of orofacial clefts remains controversial. An important approach to study CL/P genetics is to evaluate genes known to contribute to syndromic forms of CL/P. They may be useful to demonstrate a significant overlap between syndromic and non-syndromic CL/P. Thus, we initiated our study on van der Woude syndrome (VWS, MIM #119300), an autosomal dominant disorder in which lower pits and occasional hypodontia are the only features distinguishing the disorder from isolated clefts. *Interferon regulatory factor-6 (IRF6)* gene, localized on 1q32.2, was recently shown to harbor mutations in patients with van der Woude and/or popliteal pterygium syndrome (PPS, MIM #119500). We screened *IRF6* for possible mutations in 22 families with VWS or PPS. Causative mutations in most of these families confirm that *IRF6* is the major VWS/PPS gene. (<http://www.icp.ucl.ac.be>) (vikkula@bchm.ucl.ac.be).

Identification of novel FGFR3 mutations in patients with hypochondroplasia. *S. Heuertz, M. Le Merrer, V. Cormier-Daire, L. Legeai-Mallet, A. Munnich, J. Bonaventure.* Department of Genetics, INSERM U393, Hopital Necker, 75743 Paris, France.

Hypochondroplasia (HCH) belongs to a family of chondrodysplasias of increasing severity including achondroplasia (ACH) and thanatophoric dysplasia (TDI and II). Most of these diseases are caused by heterozygous mutations in the Fibroblast Growth Factor Receptor3 (FGFR3) gene. While a recurrent mutation (G380R) in the transmembrane domain of the receptor accounts for more than 98% of ACH cases, a recurrent mutation (N540K) in the tyrosine kinase 1 (TK1) domain is responsible for only 55-60% of HCH cases. An additional mutation in the TK2 domain (K650N/Q/T) also causes HCH but remains extremely rare. Based on these results, genetic heterogeneity of HCH has been suggested but is still debated. To test this hypothesis we have sequenced the entire coding region of the FGFR3 gene in 24 patients with clinical features of HCH but lacking the common mutations in exons 13 and 15. Five novel missense mutations in exons 3, 5, 7 and 14 were identified. One mutation occurred in a familial form of HCH and segregated with the disease. The other cases were sporadic and the single base changes were absent both in the unaffected parents and in more than 100 controls. Two of these mutations created cysteine residues in the extracellular domain of the protein and are likely to induce constitutive activation of the receptor through formation of a disulphide bond. Interestingly, patients carrying cysteine substitutions had a phenotype similar to patients with the common N540K mutation, whereas the other novel mutations were associated with relatively mild phenotypes. We conclude that the spectrum of FGFR3 mutations causing HCH is wider than originally thought, but our inability to detect FGFR3 mutations in a significant number of cases further supports the idea that HCH is genetically heterogeneous. This work was supported by the European Skeletal dysplasia Network (ESDN), grant CE-QLG1-CT-2001-02188.

Clustering of mutations in the actin-binding domain of Filamin B leads to Atelosteogenesis types I and III. D.

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Mutations in Filamin B (*FLNB*) gene produce a spectrum of osteochondrodysplasias including autosomal recessive spondylocarpotarsal syndrome, and the autosomal dominant disorders, Larsen syndrome, Atelosteogenesis types I and III (AOI and AOIII). AOI and AOIII are lethal skeletal dysplasias characterized by multiple joint dislocations, abnormally formed bones, and disharmonic ossification of the tubular bones of the hands and feet. AOI is neonatal lethal and growth plate histology shows areas of acellularity with multinucleated cells in the resting zone. AOIII is not uniformly lethal, and cartilage histology shows areas of hypocellularity, but no multinucleated cells. Individuals with AOI and AOIII are heterozygous for missense mutations in the *FLNB* gene. In ten of eleven individuals, we found that mutations clustered in the calponin homology domain 2 (CHD2) of the actin-binding domain (ABD) of *FLNB*. Two mutations (G168S and M202V) were seen independently in unrelated individuals. In one familial case, the affected child with lethal AOIII was heterozygous for the G168S missense mutation, while the mother was mosaic for the mutation and manifested Larsen syndrome. This indicates that Larsen syndrome can result from somatic mosaicism for a *FLNB* structural mutation. In all cases of the AO, the predicted amino acid substitutions were in residues conserved among the three orthologous filamins and other similar actin-binding proteins (dystrophin, -spectrin, and -actinin-4). Although the mechanism by which these mutations produce disease is unknown, cultured fibroblasts from AOI/AOIII cases showed occasional multinucleated cells, reminiscent of chondrocytes in the growth plate, suggesting that one consequence of the mutation is a defect in cell division. Clustering of lethal AOI/AOIII mutations in the ABD of *FLNB* implicate a consistent mechanism by which the sequence and structure of this domain is essential for normal skeletogenesis.

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Evaluating *ARSE*, an orphan enzyme in cartilage development, in which defects cause CDPX1. *M. Nino, S. Scheper, L. Chen, N. Braverman.* The McKusick-Nathans Institute of Genetic Medicine, Department of Pediatrics, Johns Hopkins Hospital, Baltimore, MD.

X-linked chondrodysplasia punctata (CDPX1), is characterized by abnormal bone and cartilage formation, midface hypoplasia, respiratory difficulties, and hearing loss. It is one disorder in a heterogeneous spectrum associated with epiphyseal stippling (chondrodysplasia punctata) on X-ray. Interestingly, the majority of these conditions are associated with defects in enzymatic pathways involving phospholipid synthesis, sterol metabolism, and vitamin K metabolism. CDPX1 is due to defects in the *ARSE* gene, which encodes the Golgi enzyme, arylsulfatase E (*ARSE*)¹. Although *ARSE* is expressed in many tissues, its function remains elusive. CDPX1 has been reported in over 100 patients, but relatively few have been studied at a molecular level. We are developing this project to improve the evaluation of patients with chondrodysplasia punctata, to offer molecular diagnosis of CDPX1 and to determine the functions of *ARSE* in normal skeletal development.

ARSE is located at Xp22.3 and spans 29.46 Kb. We have designed primers and amplified all 11 exons and boundary intronic regions, which we directly sequenced. In 4 new families with CDPX1 we identified 2 unique missense alleles, I40S, in one proband and his carrier mother and, T409M, in a 2nd proband and his carrier mother. We also identified a previously reported allele, G137A², in the 3rd proband and his carrier mother. The 4th proband has evidence of a de novo rearrangement of *ARSE*. Of the 3 missense changes, only the latter occurs at a conserved amino acid position. None are reported polymorphisms. In addition, we identified an alternative *ARSE* cDNA transcript by RT/PCR analysis of liver, kidney and fibroblast tissues. The transcript remains in frame but is lacking exon 2, which encodes the signal peptide and 2 metal coordination sites. Currently, we are planning additional functional studies of *ARSE* transcripts and proteins.

Ref: 1. Franco, B., Meroni G., Parenti G. et al. (1995).Cell. 81:15-25. 2. Sheffield L.J., Osborn A.H., Hutchison W.M. et al. (1998) J Med Genet. 35:1004-1008.

A recurrent mutation in the Dyggve-Melchior-Clausen/Smith-McCort dysplasia disease gene identified in families from Guam, Argentina and Chile. R. Pogue¹, N. Ehtesham¹, G. Repetto², R. Carrero-Valenzuela³, C. Bazan de Casella³, S. Pintos de Pons³, D. Cohn^{1,4}. 1) Medical Genetics Institute, Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) Universidad del Desarrollo-Clinica Alemana, Santiago, Chile; 3) Medical Genetics Center, UNT-FM, Tucuman, Argentina; 4) David Geffen School of Medicine, UCLA.

Dyggve-Melchior-Clausen dysplasia (DMC) and Smith-McCort dysplasia (SMC) are allelic autosomal recessive osteochondrodysplasias resulting from mutations in the *FLJ90130/dymeclin* gene (Cohn et al, 2003; El Ghouzzi et al, 2003). The two conditions have identical radiographic features and cartilage histology, but are distinguished by the occurrence of developmental delay and mental retardation in DMC. It was previously demonstrated that DMC results from homozygosity or compound heterozygosity for null mutations, compatible with absence of the gene product as the cause of the disorder. SMC in two families from Guam was shown to be produced by compound heterozygosity for a missense mutation (E87K) and a splicing mutation resulting in leaky skipping of exon 8. Although it was hypothesized that the milder SMC phenotype resulted from a small amount of normal gene product derived from the exon skipping allele, we have now identified two families in which DMC was produced by homozygosity for the exon 8 skipping mutation. These data suggest the alternative hypothesis that the E87K mutation is hypomorphic with the mutant protein retaining sufficient residual function to result in SMC rather than DMC. The occurrence of this sequence change in families from diverse geographic regions may imply a mutational hotspot, but the mutation does not occur within a sequence with a known increased mutation rate, such as a CpG dinucleotide. It is also possible that the mutations have a common ancestral origin in a colonizing population such as the Spanish, who were among the first explorers to visit all three countries during the first half of the sixteenth century and contributed to the current population of all of the countries in which the exon skipping mutation has been identified thus far.

Expression and functional gene analysis in periosteal fibroblasts from Apert syndrome patients. *A.L. Sertie¹, R.D. Fanganiello¹, N. Oliveira¹, S. Cavaleiro², N. Alonso³, M.R. Passos-Bueno¹.* 1) CEGH-IBUSP,Brazil; 2) UNIFESP,Brazil; 3) FMUSP,Brazil.

Apert syndrome (AS), the most severe of the craniosynostosis syndromes, is characterized by premature closure of coronal sutures, severe syndactyly of hands and feet and mental retardation. The majority of AS patients harbor one of two gain of function missense mutations localized in the fibroblast growth factor receptor 2 (FGFR2) region that forms the recognition site for FGF ligands: S252W and P253R, both related to ligand-binding inespecificity and increased ligand affinity. FGFRs are transmembrane tyrosine kinase receptors that play critical roles in skeletal development. Ligand activation of FGFRs initiates signal transduction through a diverse array of signaling pathways, which can lead to cell's proliferation, growth inhibition, or differentiation. Although it is known that AS osteoblast mutations cause overexpression of PKC pathway, premature differentiation and increased ossification, the abnormal FGFR2 signaling is not fully defined. We aim to gain further insight into signaling pathways and transcription factor networks derived from FGFR2 activation, as well as to identify genes involved in the molecular mechanisms of AS. As an experimental model we are examining the expression profile of primary cultured fibroblasts from the coronal suture periosteum (multipotent cells with the potential to differentiate into osteoblasts) of three AS patients (S252W) and comparing them to those of three wild type cultured periosteal fibroblasts. A set of 6 oligonucleotide arrays (CodeLink system) representing 20,000 human genes are being used. Differences in expression patterns of genes such as TGFB1, INTERLEUKIN 1, and in the transcription factors TWIST, MSX2, STAT1, DLX5 and SOX4, some of them previously showed in cells from AS patients, were observed. To further investigation we intend to use Real Time-PCR and RNA interference experiments. These studies may lead to a better understanding of the pathophysiology of AS, and may provide basis for future attempts to develop novel and effective therapies in order to ameliorate the clinical features.(FAPESP/CEPID - fanga@sti.com.br).

HOXD10 gene screening in cases with Congenital Vertical Talus (CVT) or Charcot-Marie-Tooth (CMT) Disease.

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We have recently reported a mutation in a five generation Upstate (northern) New York Caucasian family of Italian descent with both Congenital Vertical Talus (CVT) and cavo-varus foot deformity consistent with Charcot-Marie-Tooth disease (CMT). Abnormal foot development segregated with an autosomal dominant mode of inheritance in this family. Twelve individuals had bilateral CVT, two individuals had bilateral CMT and two individuals had both CVT and CMT, one of whom had CVT in one foot and later developed CMT in the other. Whole genome linkage analysis using Affymetrix 10K Arrays defined a 7 Mb critical region on chromosome 2q31. Candidate gene sequencing of six homeodomain containing genes known to have a role in limb development (HOXD9, HOXD10, HOXD11, HOXD12, HOXD13, and EVX2) lead to the detection of a single missense mutation (M319K) in the HOXD10 gene (Shrimpton et. al.). This mutation is at a highly conserved base in the homeodomain and is located in the recognition helix at a position that projects into the major groove of the binding site on the target DNA. A LOD score greater than 6 was obtained from a linkage analysis on this family.

No additional mutations were detected from sequencing the HOXD10 gene in other cases which include: a. Non-familial CVT associated with skeletal muscular atrophy; b. CVT, developmental hip dysplasia and maternal clubfoot; c. CVT, developmental hip dysplasia and possible neuropathy; d. Probable CMT.

Shrimpton AE et al (2004) A HOX Gene Mutation in a Family with Isolated Congenital Vertical Talus and Charcot-Marie-Tooth Disease. *AJHG* 75:92-96. *HOXD10* *HOXD10*.

Balanced (9;11) translocation in a patient with non-syndromic craniosynostosis disrupts the SOX6 gene (11p15) and a conserved non-transcribed region (9q32). A. Tagariello¹, R. Heller², A. Greven³, V. Kalscheuer⁴, T. Molter¹,

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Craniosynostosis is a congenital developmental disorder involving premature fusion of cranial sutures resulting in an abnormal shape of the skull. While significant progress has been made in understanding the molecular basis of syndromic craniosynostosis, little is known about the various forms of non-syndromic craniosynostosis. Here we report on a male infant with non-syndromic craniosynostosis presenting at birth with Crouzon-like brachycephaly, proptosis, midfacial hypoplasia and low set ears. Three-dimensional cranial computer tomography showed fusion of the lambdoid sutures and distal part of the sagittal suture with a gaping anterior fontanelle. Mutations in the genes for *FGFR1-3* were excluded. Standard chromosome analysis revealed a de novo balanced translocation t(9;11)(q32;p15). We have cloned the DNA fragment containing the chromosome 9 and 11 breakpoints. The breakpoint on chromosome 11p15 disrupts the *SOX6* gene, a gene known to be involved in skeletal growth and differentiation processes. Consequently, we have screened the complete *SOX6* gene in 102 patients with non-syndromic craniosynostosis. No causative mutation was found. Nevertheless, we can not rule out that the translocation may have lead to a truncated *SOX6* protein exhibiting a dominant negative effect. The breakpoint on chromosome 9 is located in a region without any known or predicted gene but, interestingly, disrupts patches of evolutionary highly conserved non-coding, non-transcribed DNA. Assuming a regulatory function of these sequences, we suggest that the translocation could have led to a dysregulation of flanking genes on chromosome 9 or 11 involved in cranial suture formation. Candidate genes have been evaluated.

Maffucci Syndrome and Olliers Disease are Not Allelic Disorders. V. Wouters¹, O. Enjolras², J.B. Mulliken³, C. Castellan⁴, L.M. Boon^{1, 5}, M. Vikkula¹. 1) Laboratory of Human Molecular Genetics, Christian de Duve Institute & Université catholique de Louvain, Brussels, Belgium; 2) Département de Dermatologie, Hôpital Lariboisière, Paris, France; 3) Division of Plastic Surgery, Childrens Hospital and Harvard Medical School, Boston, MA, USA; 4) Laboratory of Cytogenetica, Bolzano, Italy; 5) Center for Vascular Anomalies, Division of Plastic Surgery, Cliniques Universitaires St-Luc, Université catholique de Louvain, Brussels, Belgium.

Maffucci syndrome is characterized by multiple cutaneous venous-like anomalies (previously called cavernous angioma or hemangioendothelioma) in association with multiple enchondromas similar to those in Olliers disease, and a high incidence of malignancy. This rare congenital disorder is not inherited and has no sex prevalence bias. Severity of the disorder comes from the development of extreme and often grotesque deformation and, a 40% risk of cancerous transformation, especially to chondrosarcoma. About 200 cases have been reported. Our hypothesis is that Maffucci syndrome belongs to the group of contiguous gene syndromes, in which germline, or somatic deletion of several genes creates the combined phenotype. We have collected clinical data and/or blood/tissue samples from 37 Maffucci patients. Recently, two Olliers disease patients were discovered to carry a mutation (R150C) in the PTHR1 (parathyroid hormone receptor-1) gene (Hopyan *et al.*, 2003). One was somatic and the other germline. On the basis of these results, we carried out a germline screening and a somatic screening for *PTHRI* gene for Maffucci and Ollier patients by SSCP (Single Strand Conformation Polymorphism) and HD (Heteroduplex) techniques, combined with sequencing analysis. No mutation was identified in the *PTHRI* gene in Maffucci patients. This suggests that *PTHRI* gene is not the Maffucci syndrome gene and thus, despite their clinical overlaps, Maffucci syndrome and Olliers disease are not allelic disorders. (vikkula@bchm.ucl.ac.be) (<http://www.icp.ucl.ac.be/vikkula>).

Construction of a rat model for clubfoot malformation and initial analysis of diseases musculature proteome. H.

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Clubfoot (talipes equinovarus) is a common type of congenital malformation for which complex segregation analyses have suggested a genetic etiology. We have explored to construct a rat model for the disease using all-trans retinoic acid (ATRA) and analyze the variations in the relevant proteomes. Various doses of ATRA were given intra-gestically to Wistar rats on gestational day 10. Rat fetuses were removed through laparotomy on day 21, with proteins extracted and analyzed. Results: 1) Clubfoot malformation, together with other external abnormalities, were found among all experimental groups, with frequencies increasing with the dosage (11.29%, 15.28%, 60.98% and 68.18%, respectively, for 120, 130, 135 and 140 mg/kg body weight group). The highest incidence for isolated clubfoot malformation (29.27%) was seen in 135mg/kg group. 2) Analysis of the spinal cord, vertebrae and hind lower leg musculatures from abnormal rats showed significantly increased level of apoptosis ($P < 0.01$). 3) Total proteome of musculatures from hind lower legs of fetus rats were separated through 2-D gel electrophoresis. Approximately 600~1000 protein spots (mainly distributed within the range of pH 5~7 and 20~100 kD) were detected, respectively, on Coomassie Brilliant Blue or silver staining. 4) Compared with those of the normal controls, samples from model rat fetus showed gains and losses, in addition with varied strength (for 5-10 times or more) in a proportion of protein spots. However, only a few spots selected for mass spectrometry analysis had acquired consistent peptide mass fingerprints (cross-checked with PeptIdent, MS-Fit and Mascot). Comparing normal and abnormal proteomes has so far shown loss of slow skeletal muscle troponin T, significantly decreased X-linked inhibitor of apoptosis and gained expression of carboxylesterase AY034877 in the model rats.

Genetic variation in the TSC1 and TSC2 genes in 24 TSC families from India. *M. Ali¹, M. Markandaya¹, S.C. Girimaji², A.K. Shukla³, S. Sachhidanand⁴, A. Kumar¹.* 1) MRDG, Indian Institute of Science, Bangalore, India; 2) Department of Psychiatry, NIMHANS, Bangalore, India; 3) Department of Radiology, KIMS, Bangalore, India; 4) Department of Dermatology, Bangalore Medical College, Bangalore, India.

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder with loci on chromosome 9q34.3 (TSC1) and chromosome 16p13.3 (TSC2). Genes for both loci have been isolated and characterized. Clinical symptoms of TSC include cortical tubers, subependymal nodules, mental retardation, seizures, autism, shagreen patches and angiofibromas on the skin, cardiac rhabdomyomas, retinal hamartomas, and angiomyolipomas in the kidneys. Several mutations have been reported in both TSC genes in patients mainly from the western and Japanese populations. However, there is no report on the mutation analysis of TSC genes in patients from the Indian population. We report here the mutational analysis of the TSC1 and TSC2 genes in 24 TSC families from India. Using PCR-SSCP and DNA sequence analyses, we have screened all 21 coding exons of the TSC1 gene and all 41 coding exons of the TSC2 gene in seven familial and 17 sporadic TSC cases. We have also sequenced promoters of both the TSC genes in 24 probands. We have identified a total of 12 mutations. Of these, seven mutations are novel. We have identified a single previously known deletion in the TSC1 gene. Of 11 mutations identified in the TSC2 gene, 3 are deletions, 2 are insertions, 3 are missense, 2 are splice site and 1 is a nonsense mutation. In addition, we have also detected three and eight variants/polymorphisms in the TSC1 and TSC2 genes respectively. Of these, three are novel SNPs. There was no correlation between the types of mutations (missense, nonsense, etc.) and the severity of the disease. As observed in the western and Japanese populations, the mutations were scattered across the TSC2 gene. DNA sequence analysis of promoter regions of both TSC genes in 24 families did not show any variation. (This work was financially supported by a grant from DBT, New Delhi to AK and SCG and a CSIR JRF to MA).

Molecular analysis of the PRKCG gene in Portuguese patients with spinocerebellar ataxia. *I. Alonso*^{1,2}, *C. Costa*³, *P. Coutinho*⁴, *I. Silveira*^{1,2}, *J. Sequeiros*^{1,2}. 1) Unigene, IBMC - Univ Porto, Porto, Portugal; 2) Lab. de Genética Médica, ICBAS, Univ. Porto; 3) Hospital Fernando da Fonseca, Amadora; 4) Hospital de São Sebastião, Feira; PORTUGAL.

Recently, mutations have been found in exon 4 of the PRKCG gene in families with spinocerebellar ataxia type 14 (SCA14), characterized by progressive incoordination of gait and limbs and by speech and eye-movement disturbances. The PRKCG gene encodes protein kinase C, a member of a family of serine/threonine kinases, which plays a role in diverse cellular processes such as signal transduction, synaptic transmission, cell proliferation and differentiation. Mutation screening in the PRKCG gene was performed in 366 Portuguese unrelated patients with ataxia. We ascertained 103 apparently autosomal dominant, 41 autosomal recessive families and 222 isolated cases with ataxia; 325 were of Portuguese origin, while 41 were from southern Brazil. Clinical manifestations in these patients included progressive gait ataxia, with or without associated symptoms, such as epilepsy, seizures, mental retardation or tremor. Mutation detection was carried out by PCR amplification, followed by SSCP and sequencing analysis. In one large family, a C-to-G heterozygous transversion in nucleotide 303 was detected, resulting in a new histidine-to-glutamine change at codon 101, located in Cys2, a cystein-rich region in the protein regulatory domain. This mutation was not detected in 400 chromosomes from a Portuguese control population. The His101 is evolutionarily conserved among PKC isozymes. No mutations were found in exon 4 in the remaining families or isolated patients. These results indicate that this and previously described SCA14 mutations are very rare.

Founder Effect in French Canadian Families with a Deletion Mutation in the CHAC Gene. *E. Andermann¹, A. Jansen¹, A. Al-Asmi¹, C. Dobson-Stone², A. Monaco², A. Lang³, F. Robert⁴, A. Badhwar¹, S. Mercho¹, F. Dubeau¹, A. Danek⁵, F. Andermann¹.* 1) Neurology & Human Genetics, McGill Univ, Montreal, PQ, Canada; 2) Monaco Group, Wellcome Trust Centre for Human Genetics, Oxford, UK; 3) Movement Disorder Clinic, Toronto Western Hosp., ON, Canada; 4) North Bay Genetics Clinic, ON, Canada; 5) Neurologische Klinik und Poliklinik-Groshadern, Klinikum der Univ. Munchen, Germany.

Background: Chorea-acanthocytosis (CHAC; OMIM 100500) is a neurodegenerative disorder characterized by the gradual onset of involuntary movements, dysarthria, areflexia, seizures and dementia, and the presence of acanthocytes. In the majority of CHAC families, the disease is inherited as an autosomal recessive trait due to mutations in the CHAC gene on chr 9q21. **Methods:** We have studied 4 French-Canadian kindreds. Medical and family histories were obtained. Biochemical tests, blood smears for acanthocytes, EEG, video-telemetry, MRI, volumetric and neuropsychological tests were performed. Bloods were collected for DNA studies in 75 individuals. **Results:** 11 patients in 6 sibships had clinical features of CHAC. 4 of the 6 sibships had clear parental consanguinity. 7 patients presented with epilepsy (6 temporal), years before developing involuntary movements including chorea, dysarthria, orofacial dyskinesia and unusual tics. The patients had mood disorders and slowly progressive cognitive and memory dysfunction. EEG confirmed temporal epileptic abnormalities. MRI of the brain showed caudate atrophy and abnormal signal in the basal ganglia. Peripheral blood smears showed acanthocytosis in all patients. All 4 kindreds were homozygous for a deletion mutation spanning exons 70-73 of the CHAC gene, and shared a common haplotype in the region. **Conclusion:** We describe four French-Canadian kindreds, three of which manifested familial mesial temporal lobe epilepsy as a presenting feature, delaying the diagnosis of CHAC. Parental consanguinity, the finding of a common haplotype and a shared deletion mutation, all suggest a founder effect. CHAC represents the first gene mutation associated with the clinical features of familial temporal lobe epilepsy, to our knowledge.

Parkin gene analysis in late onset autosomal recessive Parkinsons disease. *F. Annesi, D. Civitelli, E.V. De Marco, P. Spadafora, I.C. Ciro' Candiano, P. Tarantino, S. Carrideo, F.E. Rocca, G. De Cicco, G. Annesi.* Inst of Neurological Sciences, National Research Council, Mangone, Cosenza, Italy.

Early onset (>45 years) Parkinson's disease (EOPD) is an autosomal recessive neurodegenerative disorder characterized clinically by early onset of typical signs of parkinsonism. Three loci have been associated with early-onset, autosomal recessive parkinsonism: Parkin (PARK 2), DJ-1 (PARK 7), PINK 1 (PARK 6). The parkin gene, located on chromosome 6q25.2-27, contains 12 exons spanning 1.53 Mb and encodes an E2-dependent E3 ubiquitin protein ligase, that has a role in the proteasome-mediated degradation of target substrates. To date, a variety of mutations in the parkin gene have been reported, including exonic rearrangements (deletions and multiplications) and several missense mutations. **Material and Methods** For evaluating the role of the parkin gene in late-onset Parkinsons disease (LOPD), we performed a molecular screening of the 12 coding exons in 23 patients from 18 families from southern Italy, with probable autosomal recessive Parkinsons disease and an age at onset of 45 years or older. The conventional mutational analysis included polymerase chain reaction (PCR) and sequencing of the PCR products of the 12 exons on both strands. To identify exon rearrangements we performed gene dosage analysis using real-time PCR 7900 HT-SDS (Perkin Elmer-Applied Biosystems). We did not find any parkin mutations either by the conventional mutational analysis or with real-time PCR in all the subjects with late-onset PD. Our results suggest that parkin gene do not play an important role in development of LOPD.

E163L homozygous DJ-1 mutation in a family from southern Italy with amyotrophic lateral sclerosis-parkinsonism- dementia complex. *G. Annesi¹, G. Savettieri², P. Tarantino¹, F. Annesi¹, D. Civitelli¹, M. D'Amelio², P. Aragonese², I.C. Ciro' Candiano¹, B. Fierro², T. Piccoli², V. La Bella², F. Piccoli², E.V. De Marco¹, S. Carrideo¹, M. Zappia³, A. Quattrone^{1,3}.* 1) Inst Neurological Sci, National Research Council, Cosenza, Italy; 2) Dept of Neurology, Ophthalmology, Otorhinolaryngology and Psychiatry, University of Palermo, Italy; 3) Inst of Neurology, University Magna Graecia, Catanzaro, Italy.

To describe two patients with familial amyotrophic lateral sclerosis-parkinsonism-dementia complex and a novel mutation in the DJ-1 gene. Three siblings from a family having consanguineous parents were examined. In two patients and in three healthy relatives (the patients mother and two siblings), DNA samples were extracted by standard methods. The entire DJ-1 gene open reading frame was amplified and directly sequenced by means of forward and reverse primers. All the patients presented a syndrome characterized by akinetic-rigid parkinsonism moderately responsive to dopaminergic drugs, muscle weakness due to upper and lower motor neurons involvement with EMG findings of denervation, and cognitive impairment. The onset of the disease was at age of 24, 35 and 36 years old, respectively. Symptoms slowly progressed and one patient died at age 43 years old, after 7 years from the onset of the disease. Sequencing analysis of the DJ-1 gene in two patients showed an homozygous point mutation in exon 7 G-A at position 3385 nt (ref ALO34417) resulting in the substitution of a highly conserved E (glutamic acid) by a L (lysine) at position 163 of the DJ-1 protein. The patients relatives showed the E163L mutation in the heterozygous state. This mutation in homozygous state showed complete segregation with the disease in the family and it was not found in 300 normal chromosomes. We have identified a E163L homozygous point mutation in exon 7 of the DJ-1 gene in a family with autosomal recessive amyotrophic lateral sclerosis-parkinsonism-dementia complex. Our findings add further evidence on the pathogenic role of DJ-1 gene in early-onset familial parkinsonism and expand the clinical spectrum of the DJ-1 related disorders.

Refining the Marinesco-Sjögren syndrome locus on 5q31. A.-K. Anttonen^{1,2,3}, I. Mahjneh⁴, B. Udd^{1,5}, H. Somer⁶, M. Somer⁷, A.-E. Lehesjoki^{1,2}. 1) Dept of Medical Genetics, and Folkhälsan Institute of Genetics, Univ of Helsinki, Finland; 2) Neuroscience Center, Univ of Helsinki, Finland; 3) Clinical Genetics Unit, Helsinki Univ Central Hospital, Finland; 4) Dept of Neurology, Pietarsaari Hospital, Finland; 5) Neurology Dept, Vaasa Central Hospital, Finland; 6) Dept of Neurology, Univ of Helsinki, Finland; 7) The Family Federation of Finland, Helsinki, Finland.

The Marinesco-Sjögren syndrome (MSS) is a rare disorder with autosomal recessive inheritance. The main features of MSS are progressive myopathy, cerebellar ataxia, bilateral cataracts, hypergonadotrophic hypogonadism, and mild to moderate mental retardation. Recently patients with typical MSS features were linked to 5q31. Another locus for MSS with myoglobinuria has been localized on 18qter. We identified and clinically characterized a large inbred Finnish family with five patients, and two pairs of affected siblings. A candidate locus approach was undertaken to define the molecular genetic background of MSS in these families. Clinical information of the patients was gathered from medical records, and all the patients alive were examined by one clinician. Microsatellite markers were tested to evaluate the previously reported loci on 5q31 and 18qter. Haplotypes spanning the candidate regions were constructed and two-point LOD scores were calculated with MLINK program. All patients had classical MSS with generalized muscular weakness and hypotonia. Patients showed ataxia, bilateral cataracts, nystagmus, and dysarthria. Muscle weakness and wasting was most marked in the lower limb-girdle with both proximal and distal muscles involved. All patients had hypergonadotrophic hypogonadism and mild mental retardation. No linkage to markers on 18qter was detected, but significant linkage was observed between MSS and markers on chromosome 5q31. A maximum two-point LOD score of 6.55 (at =0.000) was obtained with marker *D5S414*. All the Finnish patients shared a homozygous, common haplotype over a subset of markers around *D5S414*. These data suggest that MSS in Finnish patients is caused by homozygosity of a founder mutation of an as yet unidentified gene on chromosome 5q31.

Two mutations in the *HSN2* gene explain the high prevalence of HSANII in French-Canadians. B. Brais¹, K. Roddier¹, T. Thomas¹, G. Marleau¹, A.M. Gagnon¹, M.J. Dicaire¹, A. Saint-Denis¹, I. Gosselin¹, A.M. Sarrazin², A. Larbrisseau³, M. Lambert³, M. Vanasse³, D. Gaudet⁴, G.A. Rouleau⁵. 1) CRCHUM, Univ. de Montréal, Montreal, Quebec, Canada; 2) Centre Hospitalier Régional de Lanaudière, Saint-Charles-Borromée, Quebec, Canada; 3) Hôpital Ste-Justine, Univ. de Montréal, Montreal, Quebec, Canada; 4) Centre de médecine génique communautaire de l'Univ. de Montréal, CH de la Sagouamie, Saguenay, Canada; 5) McGill Health Centre, McGill Univ., Montreal, Quebec, Canada.

Hereditary sensory and autonomic neuropathy type II (HSAN II, MIM 201300) is a rare childhood recessive neuropathy usually diagnosed in childhood or the early teens. It was first clearly described in 1973 in a French-Canadian (FC) kinship. The HSANII locus and the *HSN2* mutated gene have been uncovered. Through treating physicians we recruited 17 HSANII cases belonging to 13 families. Following informed consent, we extracted DNA of 17 patients and their relatives. Further to the identification of the responsible gene, we sequenced the whole *HSN2* gene in order to search for mutations. We have uncovered the largest international cluster ever described. They all originated from southern Quebec. Haplotype analysis predicted that two distinct mutations were responsible for HSANII in FC. Two distinct loss-of-function mutations were identified, both are predicted to lead to truncations of the protein. The higher prevalence of this condition in Quebec appears to be due to a relatively high carrier frequencies for both mutations. The following genotypes were uncovered in the different families: 58.3% of cases were homozygote for mutation 1 and 8.3% for mutation 2, and 33.3% were compound heterozygotes. 75% of chromosomes carried mutation 1 and 25% mutation 2. The comparison of clinical variables does not suggest any difference in phenotype between carriers of the two mutations. HSANII is more frequent in the FC population due to higher carrier rates for two distinct *HSN2* founder mutations. This is the first example of a FC recessive disease clearly more associated with a regional population of Southern Quebec. The uncovering of *HSN2* mutations will lead to better genetic counseling.

***FMRI* gene premutation is a frequent genetic cause of late-onset sporadic cerebellar ataxia.** A. Brussino¹, C. Gellera², A. Saluto¹, C. Mariotti², C. Arduino¹, B. Castellotti², N. Migone¹, F. Taroni², A. Brusco¹. 1) Genetics Biology & Biochem, University of Torino & Operative Unit of Medical Genetics, San Giovanni Battista Hospital, Torino, Italy; 2) Dept. Biochemistry and Genetics, National Neurological Institute IRCCS "Carlo Besta", Milano, Italy.

Premutation in the fragile-X (*FMRI*) gene has been associated with an increased incidence of premature ovarian failure in females and with a neurodegenerative disorder in males known as Fragile-X Tremor Ataxia Syndrome (FXTAS). We have collected a large cohort of 275 males affected by a sporadic form of spinocerebellar ataxia, referred for genetic analysis of *SCA* genes. The presence of pathological expansions was excluded in the *SCA1* and *SCA2* genes - accounting for over 90% of familiar *SCA* in Italy - together with *SCA3*, *SCA6*, *SCA7* and *FRDA1* gene expansions whenever a suggestive phenotype was present; age at onset ranged from 20 to 82 yr (mean 48.3 yr 14.5 S.D.). A novel fluorescent PCR analysis of the *FMRI* CGG repeat (able to detect up to 300-CGGs) was used to screen our patients: six were found carriers of a premutated allele (range 83-109 repeats). Clinical/neuroradiological findings were consistent with the FXTAS syndrome, and age at onset ranged from 53 to 69 yr. MRI showed cerebellar atrophy and T2-hyperintense middle cerebellar peduncles. Our results show that FXTAS is a frequent genetic cause of idiopathic ataxia accounting for 2.2% (6/275) of adult-onset cases. The frequency raises up to 4.2% (6/143) if one considers only cases with age at onset above 50 yrs, suggesting that it is a frequent genetic cause of late-onset sporadic ataxia. Since the analysis of the *FMRI* premutation is simple and rapid, genetic testing the CGG repeat number could be suggested as the first-choice investigation in all cases of late-onset idiopathic ataxia.

Mutations in the NHLRC1 gene are common in patients with Laforas progressive myoclonus epilepsy. D. Civitelli¹, F.E. Rocca¹, I.C. Ciro Candiano¹, R. Biondi², V. Sofia², G. Milana³, N. Cutuli³, E.V. De Marco¹, F. Annesi¹, P. Tarantino¹, S. Carrideo¹, G. De Cicco¹, G. Annesi¹. 1) Inst of Neurological Sciences, National Research Council, Mangone, Cosenza, Italy; 2) Institute of Neurology and Pediatrics, University of Catania, Italy; 3) Laboratory of Molecular Genetics, Dept of Services, University of Catania, Italy.

Lafora's progressive myoclonus epilepsy is an autosomal-recessive disorder caused by mutations in the EMP2A gene, which is located on chromosome 6q24. An ulterior causative gene for Laforas disease was recently identified on chromosome 6p22, termed NHLRC1 or EPM2B. It is a single-exon gene, encoding a putative E3 ubiquitin ligase (malin). In this study, we screened NHLRC1 gene in three patients. Examined probands are three Sicilian patients who received a diagnosis of LD after a comprehensive clinical and laboratory investigation. In two of them, age at onset of LD was 14 years, in the remaining patient LD started at the age of 11 years. Sequencing analysis of EPM2A did not reveal any pathogenic variant in all three patients. Then, we went on sequencing the NHLRC1 gene. We designed six primers pairs, to obtain six overlapping PCR fragments, spanning the whole NHLRC1 exon, including non-coding sequences. Amplified fragments were directly sequenced. In one patient we found a novel homozygous nonsense mutation (G199T, giving rise to a stop codon at aminoacid residue 67). The second patient carried a compound heterozygous for two mutations (C205G causing the P69A substitution, and G838A resulting in the E280K change). The last patient carried the heterozygous E280K mutation and a known polymorphism (C332T leading to the P111L substitution). All the mutations but the latter polymorphism were not found in 100 control chromosomes. The results of the present study provide evidence that mutations in the NHLRC1 gene may be a common cause of LD in populations of Mediterranean origin. Supported by Ministero della Sanit Progetto biennale Genetica dell'Epilessia.

Investigation for intragenic rearrangements of the PLP1 gene in patients with dysmyelinating leukodystrophies using MAPH. *P. Combes¹, C. Vours-Barriere¹, MN. Bonnet-Dupeyron¹, F. Gauthier-Barichard¹, J. Armour², O. Boespflug-Tanguy¹.* 1) UMR 384, Medecine Faculty, Clermont-Fd, France; 2) Institut of Geneticq, University of Nottingham, UK.

The PLP1 gene is known to be mutated in the X-linked myelination disorders, Pelizaeus-Merzbacher Disease (PMD) and Spastic Paraplegia type 2 (SPG2). The most common PLP1 mutation in PMD is a large duplication including the whole gene, whereas large deletions and punctual mutations can be responsible for PMD or SPG2 phenotypes. Semi quantitative PCR (only on 1 or 2 exons) has been used to quantify PLP1 gene copy number; looking only for large rearrangements. In the aim to search for small intragenic PLP1 rearrangements in patients presenting classical forms of PMD or SPG2 and not diagnosed as PLP1 mutated, we developed the MAPH (Multiplex Amplifiable Probe Hybridization) technique using 13 PLP1 specific probes distributed along the gene (2 promoter, 4 intronic and 7 exonic regions probes). This technique relies on the quantitative recovery and amplification of nucleic probes after hybridization to immobilized DNA. In a first approach, DNAs from 20 patients and 15 carrier mothers already diagnosed with duplications have been used to validate the technique and have shown that all the duplications encompass the entire PLP1 gene. Then, a total of 340 patients presenting with dysmyelinating leukodystrophies (including classical PMD, SPG2 and PMD or SPG like phenotypes) were screened. Whereas no duplications could be detected, two partial deletions of the PLP1 gene were identified. One deletion involves the 5 part of the gene (promoter to part of intron 1); the other one is a 3 gene deletion (exon 6 to 7). As it has been demonstrated for the DMD gene, MAPH is a powerful and cost effective tool to screen cohorts of patients for small intragenic or large genomic rearrangements.

Ataxia with oculomotor apraxia in a family from southern Italy: a clinical and genetic study. *E.V. De Marco, P. Spadafora, D. Civitelli, F. Annesi, S. Carrideo, I.C. Cir Candiano, P. Tarantino, F.E. Rocca, G. De Cicco, G. Annesi.* Inst of Neurological Sciences, National Research Council, Mangone , Cosenza, Italy.

Ataxia with oculomotor apraxia type 1 (AOA1) is caused by mutations in the aprataxin gene (APTX) which maps to chromosome 9p13 and codes for a nuclear protein involved in single-strand DNA break repair. Here we report on a family from southern Italian in which three members were affected by AOA1. There were three affected members: one 30-year-old man and two women of 39 and 37 years of age respectively. In all of them, symptoms started around the age of 15 months with progressive cerebellar ataxia. Important, the brother and the 39-year-old sister had also had repeated febrile convulsions around the age of 8 and 18 months respectively. From the time of the onset, the course of the disease was severe with appearance of cognitive impairment and all patients became wheelchair-bound. Oculomotor apraxia and severe axonal sensorimotor neuropathy were present in all patients. Laboratory investigation showed hypoalbuminaemia and hypercholesterolaemia; brain MRI revealed cerebellar atrophy. Genetic analyses for spinocerebellar ataxia types 1, 2, 3, 6, 7, 8, 12 and 17, for hereditary dentatorubropallidolusian atrophy, for Friedreich's ataxia and for ataxia-telangiectasia excluded the diagnoses of these disorders. Screening for mutations in the APTX gene was performed by sequencing exons 4, 5, 6 and 7. We found the patients bearing the homozygous W279X nonsense mutation which produces a protein truncated at aminoacid 279. As expected, their parents were both heterozygous for the mutation. We have described here the third Italian family with molecularly proven AOA1. Also our family carried a W279X substitution which is the most common mutation in Portuguese families.

ALS2/alsin detection in 26 patients with ALS2, JPLS, IAHS2 phenotypes. *E. Eymard-pierre¹, K. Yamanaka², P. Combes¹, D. Cleveland², E. Bertini³, O. Boespflug-Tanguy¹, and the European SPATAX consortium⁴.* 1) INSERM U 384, clermont-fd, France; 2) Ludwig Institute for Cancer Research, UC at San Diego, USA; 3) Bambino Gesu Childrens Hospital, Roma, Italy; 4) A. Durr, Inserm U289, Paris, France.

Non-sens mutations in the ALS2 gene have been reported in autosomal recessive forms of juvenile Amyotrophic Lateral Sclerosis (ALS2), juvenile primary lateral sclerosis (JPLS), and infantile ascending hereditary spastic paralysis (IAHS2). These 3 disorders are characterised by a progressive ascending spastic paralysis due to a retrograde degeneration of the upper motor neurons with (ALS2) or without (JPLS, IAHS2) involvement of the lower motor neurons. Using an antibody against RCC1 domain of the ALS2 protein, we demonstrated that ALS2 protein can be detected by western blot from lymphoblastes or fibroblasts and is absent in patients with ALS2 mutations. We screened for ALS2 a cohort of 26 families with primary involvement of the upper motor neurons. The ALS2 locus was excluded by haplotype analysis in 6 families. In the remaining 20 families, presence of the ALS2 protein was detected by western blot of lymphoblasts. ALS2 protein was detected in 13 patients and absent in 7. In only four out of these seven ALS2 negative patients, mutations were detected by direct sequencing of cDNA. We will quantify in these patients the ALS2 transcripts. Clinical analysis of the 35 patients demonstrated that the ALS2 positive patients tend to have a later age of onset, a slower progression, more frequent cognitive and SEP abnormalities and less frequent MRI abnormalities and display a lower motor neuron involvement in 20 percent; which was never observed in the ALS2 negative group. In conclusions, early onset, autosomic recesive forms of primitive degeneration of the upper motor neurons are genetically heterogenous. ALS2 mutations are found in only 12 percent of patients where an additionally 8 percent demonstrated an absence of ALS2 protein in lymphocytes. Therefore detection of ALS2 proteins in lymphocytes by western blot could be an interesting screening to look for ALS2 involvement in patients with ALS2, JPLS and IAHS2 phenotypes .

An expanding deletion on chromosome 14 in a family with dopa-responsive dystonia and ptosis. *K. Hedrich^{1,2}, R. Saunders-Pullman^{3,4}, K. Wiegers^{1,2}, F. Hinrichs¹, Y. Hellenbroich¹, K. Kabakci^{1,2}, V. Pulijaal⁵, K. Habermann³, L.J. Ozelius⁴, E. Schwinger¹, S.B. Bressman³, C. Klein^{1,2}.* 1) Hum. Genet., Univ. Luebeck, Germany; 2) Neurology, Univ. Luebeck, Germany; 3) Neurology, Beth Israel Medical Center, New York, NY; 4) Mol. Genet. and Neurology, A. Einstein College of Medicine, Bronx, NY; 5) Reprod. Genet., A. Einstein College of Medicine, Bronx, NY.

Dopa-responsive dystonia (DRD) is a childhood-onset movement disorder with an excellent response to L-Dopa. Most DRD is autosomal dominantly inherited with reduced penetrance and associated with mutations in the GTP cyclohydrolase I (GCHI) gene on chromosome 14q22. More than 80 different point mutations and, more recently, five different heterozygous deletions involving one or more exons have been described in GCHI. We present a three-generation family with four DRD patients and six unaffected who were tested for mutations in GCHI by sequence and quantitative duplex PCR analysis. No alteration in the GCHI gene was found by sequencing, however, we identified a heterozygous deletion of all six GCHI exons by quantitative PCR in all four affected and one unaffected individual, confirming reduced penetrance. Surprisingly, one obligate carrier, whose sister and son are both affected, did not carry this deletion. We confirmed paternity, however, haplotype analysis of the 14q22 region revealed several incompatibilities. Using 30 microsatellite markers, we demonstrated that this obligate carrier and his mother had another smaller deletion of 700kb localized downstream of GCHI. This deletion expanded independently twice in this family to a deletion of about 3600kb involving about 20 genes including GCHI. We verified these results by FISH analysis, using three different BAC clones from the RZPD (Berlin, Germany) that allowed us to distinguish between the two different deletions. Interestingly, all five carriers of the larger deletion also presented with ptosis, regardless of their DRD status. The deleted gene responsible for the ptosis remains to be identified, as do the break points of the deletions to understand the underlying mechanism of the deletion expansion.

A novel CAG/CTG repeat expansion in a patient with a progressive sensory neuropathy. *S.E. Holmes¹, J.S. Wentzell², C. Callahan¹, C.A. Ross¹, R.L. Margolis¹.* 1) Dept of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Colby College, Waterville, ME.

CAG/CTG repeat expansions cause at least 12 different movement disorders, and our data suggests that additional disorders of this type may exist. Using the repeat expansion detection (RED) assay, we identified an expanded CAG/CTG repeat in a 50 year old woman with a progressive sensory neuropathy. The father, brother, and cousin of the proband are affected with a similar disorder. All known causes of CAG/CTG repeat expansions were excluded in the proband. To identify the locus of the expansion, we used PCR to test the repeat length of 267 CAG repeats in the human genome 8 or more triplets in length. The expansion was localized to an intron of a Genscan-predicted gene, approximately 200 bp downstream of an exon which is conserved in mouse. The closest experimentally verified gene in the region (encoding a serine/threonine kinase) occurs approximately 63 kb downstream from the repeat. We examined the repeat length, using an automated genotyping protocol, in the proband, her affected cousin, 255 control individuals and 219 patients with movement disorders of unknown cause. DNA from the brother and father is currently unavailable. The length of the expansion in the proband, verified by sequencing, was 98 triplets. The proband's cousin did not have an expanded repeat, and no expansions were detected in the patient group or the control group. The repeat length ranges from 5 to 27 triplets, with 8 triplets the most common allele. Heterozygosity is 59.5%. Our results suggest that this new repeat expansion is rare and probably is not the direct cause of the phenotype in our proband. Whether the repeat contributes to the patient's phenotype, or is associated with another phenotype, remains to be determined.

Purkinje cell degeneration and 1C2 positive neuronal intranuclear inclusions in SCA8 patients. *Y. Ikeda¹, M.L. Moseley¹, J.C. Dalton¹, M.T. Su², H.M. Hsieh-Li², G.J. Lee-Chen², H.B. Clark¹, J.W. Day¹, L.P.W. Ranum¹.* 1) Inst of Human Genet, Univ of Minnesota, Minneapolis, MN; 2) Department of Life Science, National Taiwan Normal University, Taipei, Taiwan.

Spinocerebellar ataxia type 8 (SCA8) is a dominantly inherited neurodegenerative disorder caused by a CTG repeat expansion. To investigate SCA8 pathogenesis we performed neuropathological studies of two postmortem human SCA8 brains. The first patient, from the large SCA8 family we initially described, had an expansion of 110 combined repeats. Moderate-to-severe loss of Purkinje cells, mild granule cell loss, and neuronal loss with gliosis in the olivary nuclei was found, with no evidence of degeneration in other parts of the brain. The second unrelated patient had approximately 140 combined repeats. Severe loss of Purkinje cells, granule cells and inferior olivary neurons was observed. Immunohistochemical analysis using the 1C2 antibody revealed panuclear staining in selected Purkinje cells and nuclear and cytoplasmic staining in selected medullary neurons with occasional neurons having discrete intranuclear inclusions. TATA-box binding protein (TBP) staining was negative for these neurons, indicating it is unlikely that the 1C2-positive inclusions represent TBP accumulation. Similar immunohistochemical findings are also found in BAC transgenic mice that we have developed with the SCA8 expansion but not in BAC control lines. It was recently demonstrated that the 1C2 antibody recognizes both homopolymeric glutamine and leucine expansions. These results suggest the following models: 1) short ORFs in the polyleucine or the polyglutamine direction are translated, although only polyleucine encoding transcripts have been detected; 2) CUG containing SCA8 transcripts induce inclusion formation similar to the model proposed for FXTAS in which CGG containing transcripts are associated with 1C2 negative but ubiquitin positive inclusions, although CUG containing RNA foci have not been detected in SCA8 human or mouse brains. Our inclusion data provide the first molecular link between the human disease and our BAC transgenic expansion mice and indicate a pathogenic mechanism involving intranuclear inclusions.

Disease mechanism for the SOX10 extension mutation that causes a novel neurocristopathy, PCWH. K. Inoue^{1, 2}, T. Ohyama², M. Khajavi², S. Rehberg³, Y. Goto¹, M. Wegner³, J.R. Lupski². 1) Dept MR & BD Res, Ntnl Inst Neurosci, NCNP, Kodaira, Tokyo, Japan; 2) Dept Mol Hum Genet, Baylor Coll Med, Houston, TX; 3) Inst Biochem, Univ Erlangen-Nürnberg, Erlangen, Germany.

SOX10 is an essential transcription factor for development and maintenance of neural crest and myelin lineages. Individuals with *SOX10* mutations can present with multiple defects including (1) peripheral demyelinating neuropathy, (2) central dysmyelinating leukodystrophy, (3) Waardenburg syndrome and (4) Hirschsprung disease. Most *SOX10* mutations result in premature termination codons (PTCs) and cause either PCWH, a combination of all four diseases, or WS4, a combination of the latter two. Previously, we identified that mRNAs with PCWH-causing *SOX10* mutations escape the nonsense-mediated decay (NMD) pathway, leading to a stable translation of dominant-negative mutant proteins. Meanwhile, mRNAs with WS4-causing mutations trigger NMD, resulting in haploinsufficiency due to the degradation of mutant mRNAs. However, the first case of PCWH had a unique 12 bp deletion that results in an 82 amino acid extension without disruption of the wildtype SOX10 protein, and the mutation mechanism for the extension mutant still remains unknown. To investigate the mechanism by which the extension mutation causes a PCWH phenotype, we performed functional analyses. We found a diminished transcription activity of the SOX10 extension mutants. A 10 amino acid peptide in the extension was found to be critical to diminish the transcription activity. This peptide sequence affected the binding of SOX10 to its target *in cis* when present downstream of the HMG domain. Either SOX10 mutants with the 10 amino acid peptide or the peptide alone do not interfere with wildtype SOX10 function *in trans*, thus excluding dominant-negative as the mutational mechanisms at least *in vitro*. Furthermore, an addition of the peptide to SOX11 dramatically diminished its transcription activity, but not on SOX9. From these findings, we conclude that the extension mutant may behave as a gain-of-function allele to cause PCWH rather than through a dominant-negative mechanism as appears to be the case for other PCWH-causing *SOX10* mutations.

Familial Spastic Paraplegia and the ALS2 gene. A. Jansen^{1,2}, I. Meijer^{3,4}, D. MacGregor⁵, F. Gros-Louis³, G. Rouleau³, F. Andermann¹, E. Andermann^{1,2,6}. 1) Dept Neurology & Neurosurgery, McGill University, Montreal, PQ, Canada; 2) Neurogenetics Unit, Montreal Neurological Institute, Montreal, PQ, Canada; 3) Centre for Research in Neuroscience and McGill University Health Centre Research Institute, Montreal, PQ, Canada; 4) Dept of Biology, McGill University, Montreal, PQ, Canada; 5) Hospital for Sick Children and University of Toronto, Toronto, ON, Canada; 6) Dept of Human Genetics, McGill University, Montreal, PQ, Canada.

Infantile onset ascending hereditary spastic paraplegia (IAHSP) is an autosomal recessive neurodegenerative disorder with early involvement of corticospinal and corticobulbar pathways and absence of lower motor neuron signs. We present a large consanguineous Pakistani kindred with IAHSP and mild intrafamilial phenotypic variability.

Fourteen patients presented with spastic paraplegia in the lower limbs before age 2, extending to the upper limbs by the end of the first decade. Tetraplegia, anarthria and dysphagia developed in the second decade. Disease progression was slow, resulting in death by the fourth decade, with relative sparing of intellectual skills. At age 13, CT, MRI and MRS of the brain, EMG, NCV and BAER in the proband were normal. VER, SSEP and MEP were abnormal. Muscle biopsy showed atrophy of type II-B fibers. A 1bp deletion (4844delT) in exon 32 of the ALS2 gene on chromosome 2q33 cosegregated with the disease. The deletion was located at the beginning of the VPS9 domain, and functional studies demonstrate that absence of a functional VPS9 domain of alsin is sufficient to cause neurodegeneration.

The IAHSP phenotype overlaps with complicated HSP, but severe upper arm and bulbar disabilities are generally absent in the latter. ALS2 mutations were originally reported in patients with juvenile ALS (ALS2). However, muscle atrophy, fasciculations or denervation on EMG were rare. Mutations in ALS2 were also found in families with early onset primary lateral sclerosis and phenotypes closely resembling IAHSP. These findings suggest that all patients with mutations in ALS2 have the same disease, rather than IAHSP, ALS2 and juvenile PLS representing 3 different allelic disorders.

Novel mutations and a frequent mutation in Japanese patients with MLC. *N. Kanazawa¹, E. Nakagawa², S. Hayakawa³, Y. Suzuki⁴, A. Shibata⁵, M. Kubota⁶, T. Kibe⁷, F. Sakai⁸, S. Tsujino¹.* 1) Department of Inherited Metabolic Disease, National Institute of Neurosciences, NCNP, Tokyo, Japan; 2) Division of Child Neurology, National Center Hospital for Mental, Nervous and Muscular Disorders, NCNP, Tokyo, Japan; 3) Department of Pediatrics, Toyohashi East Hospital, Aichi, Japan; 4) Department of Neuropediatrics, Aichi Welfare Center for Persons with Developmental Disabilities, Aichi, Japan; 5) Department of Pediatrics, Tokyo Women's Medical University Daini Hospital, Tokyo, Japan; 6) Department of Pediatrics, Tokyo University, Tokyo, Japan; 7) Department of Pediatrics, Seirei Mikatahara Hospital, Shizuoka, Japan; 8) Department of Neurology, Kitasato University, Kanagawa, Japan.

Megalencephalic leukoencephalopathy with subcortical cysts (MLC or van der Knaap disease), is an autosomal recessive disorder clinically characterized by macrocephaly, ataxia, spasticity, and mental decline. MRI shows swollen brain with diffuse white-matter abnormalities and subcortical cysts.

After informed consent was obtained, we analyzed the *MLC1* gene of six Japanese patients with MLC, suspected by clinical symptoms and MRI findings. Two of them were homozygous for a previously-described mutation, S93L. Three were heterozygous for S93L, and the other allele is unexpressed, aberrant splicing, and unknown, respectively. The remaining one was a compound heterozygote for two novel mutations, A275E and 436GA, which leads to aberrant splicing.

Combining our data with previous reports allows us to estimate the molecular genetic basis in a total of fourteen Japanese patients. S93L was observed in 12 of 14 (85.7%) patients at least in one allele, and in 19 of 28 alleles (67.9%). Therefore, S93L appears to be fairly frequent in Japanese patients with MLC, and analysis for this mutation in DNA isolated from leukocytes would provide for an easy and precise diagnosis of this disorder in Japanese patients.

PANK2 mutations in a Japanese girl with motor and emotional problems. *I. Kondo¹, Y. Nakadoi², N. Jyofuku², T. Iwai², K. Miyamoto¹, H. Yamagata¹, Y. Tabara¹, S. Endo².* 1) Dept Medical Genetics, Ehime Univ Sch Medicine, Ehime, Japan; 2) Dept. of Pediatrics, Kagawa Children's Hospital, Kagawa, Japan.

Pantothenate kinase-associated neurodegeneration (PKAN) is a rare autosomal recessive disorder characterized by progressive dystonia, rigidity and pigmentary retinopathy. MRI is the most distinct diagnostic test in patients with PKAN and all patients with characteristic MRI finding, called the eye of the tiger sign, have pantothenate kinase 2 gene (PANK2) mutations. At present, over 50 different mutations have been reported in PANK2. However, clinical phenotypes in patients with PKAN have shown a wide spectrum even in the same family members and a genotype-phenotype association has not been established. Pathogenic findings in autopsy brains were reported in several Japanese patients with Hallervorden-Spatz syndrome before identification of disease gene, but PANK2 mutations have not been studied. We report here the first Japanese patient with PANK2 mutations. She was an only one child from unrelated healthy parents. Her birth and developmental history were normal by the age of four years. She began having walking difficulty with drop feet and rigidity at five years of age. The mother noted that the patient had night blindness at the same age. At the age of seven years of age, poor performance and emotional problems were noted at school and she was diagnosed having PKAN based on a retinitis pigmentosa and the eye of the tiger sign in her MRI findings. Molecular analyses confirmed two mutations in PANK2, 4 bp deletion in exon 3 derived from her mother and a missense mutation, E444K, in exon 6 derived from her father. This missense mutation was not detected in 104 unrelated healthy controls. Variable onset of disease and symptoms often delay the diagnosis of PKAN. In our case, symptoms might begin at four years of age and characteristic MRI findings presented at the age of seven years. Further PANK2 analysis in patients with walking difficulty and night blindness is very important for establishment of early diagnostic criteria for PKAN before school of age.

Selected mutations in ASPM and Microcephalin excluded as candidates in 6 Iranian families with microcephaly.

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Background: Primary autosomal recessive microcephaly (MCPH) is a rare, genetically heterogeneous disorder that results in a structurally normal but reduced in size, cerebral cortex and mental retardation. At least six different loci have been shown to be linked to this defect and mutations in two genes have been identified in families with MCPH. At least 23 families have mutations in the *ASPM* (abnormal, spindle-like, microcephaly-associated) gene and two families sharing a common ancestral haplotype have mutations in the *Microcephalin* gene. Most of the reported families with identified mutations were from Northern Pakistan; however, one Jordanian, one Saudi Arabian, two Yemeni, and two Dutch families were also studied. **Methods:** We have examined 6 families with MCPH from South Central Iran (Fars Province). Each of the probands has one or more affected relatives, is a result of 1st cousin marriage, and meets the classic criteria for this defect with head circumferences of 3-7 standard deviations below normal, and mild to moderate retardation. We have screened each proband for mutations in portions of the *ASPM* and *Microcephalin* genes using genomic sequencing. The analyzed regions included exon 2 of the *Microcephalin* gene and exons 15, 16, 23 and part of exon 3 of the *ASPM* gene. The exons selected for this initial mutation screen were chosen because they contain mutations that have been identified in more than one family. **Results:** We have found no genetic mutation or variation that would change the amino acid sequence or result in protein truncation in the coding regions examined; however, we have identified 2 single nucleotide polymorphisms (SNPs) present in both affected and unaffected individuals. **Conclusion:** This study expands on the currently reported group of families to include a new region of the world. Our future studies will include screening of additional exons of the *ASPM* gene where mutations have been found and linkage analysis studies to test current and/or identify additional loci for MCPH.

A novel missense mutation in the ND gene in a child with Norrie disease and severe neurological involvement. *D. Lev^{1,2}, M. Hasan², Y. Weigel³, E. Gak³, C. Vinkler¹, T. Lerman-Sagie², N. Watemberg².* 1) Inst Medical Genetics, Wolfson Medical Ctr, Holon, Israel; 2) Pediatric Neurology Unit, Wolfson Medical Ctr, Holon, Israel; 3) Danek Gartner institute for human genetics, Sheba Medical Center.

Norrie disease is an X-linked recessive disorder characterized by congenital blindness and in some cases mental retardation and deafness. Neurologic abnormalities including myoclonus have rarely been described. We report a novel mutation identified in a patient with Norrie disease and severe neurological involvement. The patient was diagnosed at the age of 6 months due to congenital blindness. At the age of 8 months he developed myoclonic movements and convulsions and his EEG demonstrated hypsarrhythmia. He has marked developmental delay. His head circumference is on the 50 centile. Mutation analysis of the ND gene (NDP) of the affected child and his mother revealed a novel missense mutation T>A at position 134 resulting in change at codon 45. Some studies have suggested that epileptic seizures and growth retardation in patients with ND are the consequence of loss of contiguous genes, because most such patients have had deletions extending beyond the Norrie locus. The fact that the affected child and his mother carry this missense mutation does not support a contiguous gene effect, but favors the pleiotropism of NDP, at least as far as the epileptic manifestations are concerned. This has been suggested by another report of 2 families with NDP missense mutation where 3 affected patients suffered from epilepsy. The severity of the phenotype may suggest the functional importance of this site as was previously suggested for a nearby missense mutation.

Familial temporal lobe epilepsy preceded by febrile seizures is not associated with mutations in GABA-A receptor subunit genes. *S. Ma¹, B. Abou-Khalil¹, G.T. Lee¹, J.S. Sutcliffe^{2,3}, J.L. Haines^{2,3}, P. Heder^{1,2}.* 1) Department of Neurology, Vanderbilt University, Nashville, TN; 2) Program in Human Genetics, Vanderbilt University; 3) Department of Molecular Physiology and Biophysics, Vanderbilt University.

The genetics of temporal lobe epilepsy (TLE) associated with febrile seizures (FS) remains incompletely understood. TLE preceded by FS has been traditionally considered an acquired disorder, even though several specific syndromes with TLE and FS have been recently characterized with autosomal dominant or digenic inheritance. FS in these patients may represent initial manifestation of inherited tendency towards seizures. Mutations in different genes encoding GABA A receptor subunits have been identified as a rare cause of some idiopathic generalized epilepsy syndromes. We investigated the possible contribution of alpha1 (GABRA1), beta1 (GABRB1), gamma 2 (GABRG2) and delta (GABRD) subunit genes to familial TLE preceded by FS. We have screened probands from 46 kindreds with a positive family history of FS and TLE, and 40 individuals with sporadic TLE preceded by FS. Each exon was screened by single strand conformation polymorphism analysis and samples showing abnormal mobility shifts were sequenced. We did not identify any disease causing mutations in these genes. We found 3 novel syndromic changes in coding sequence of GABRA1 gene, 1 novel syndromic change in GABRG2 and one novel single nucleotide polymorphism (SNP) with an insertion in a 5UTR region of GABRA1, that was present in 3 kindreds but not in 200 normal controls; however, this sequence change did not fully segregate with the disease. We also identified several novel SNPs in intronic regions of all four genes analyzed. Our preliminary results indicate that TLE preceded by FS is not caused by mutations in GABA-A receptor subunit genes. We identified several novel SNPs in coding regions that need to be further investigated as possible susceptibility factors for this type of familial epilepsy.

Identification of FOXP2 truncation as a novel cause of nonsyndromic developmental speech disorder. K.D.

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Forkhead-box transcription factors play important roles in embryogenesis. A point mutation in FOXP2 segregates with speech and language deficits in a 3 generation family (known as KE). This mutation, a G-to-A transition in exon 14, causes an Arg-to-His substitution within the DNA-binding motif. In addition, a translocation disrupting FOXP2 was found in an unrelated child with a similar phenotype. Extensive studies of children with autism and with specific language impairment failed to identify further FOXP2 coding changes. However, an overt feature of disorder in the KE family is verbal dyspraxia (problems co-ordinating mouth movements underlying speech), and, as yet, no study has fully examined FOXP2 in children with the appropriate phenotypic profile. Thus, in the present study we searched for mutations in all known FOXP2 exons in 43 probands with verbal dyspraxia. 7 probands had sibs who were also affected. All children had normal karyotype, no dysmorphic features, no mental retardation or additional diagnosis of medical/genetic disorder. A heterozygous mutation was identified in one proband, his affected sib, and their mother. This C-to-T change creates a stop codon in exon 7, yielding a dramatically truncated FOXP2 protein. Our data support the hypothesis that reduced FOXP2 dosage during neuronal development leads to disrupted speech. Moreover, they suggest that FOXP2 mutation is more frequent in children with verbal dyspraxia than in other language-related disorders.

CMT caused by EGR2 mutations: a natural history study of 10 patients. *P. Mancias*¹, *K. Szigeti*², *W. Wiszniewski*², *G.M. Saifi*², *G. Miller*³, *L. Keppen*⁴, *D.L. Daentl*⁵, *J.R. Lupski*². 1) Neurology, Univ TX Health Sci Center Med, Houston, TX; 2) Dept Genetics, Baylor Col Med, Houston, TX; 3) Dept Neurol, Baylor Col Med, Houston, TX; 4) Dept Ped and Adol Med, The Univ of S Dakota Sch of Med; 5) Shriners Hosp N Calif.

We have ascertained 10 consecutive patients with various EGR2 mutations and collected follow-up information regarding disease progression and respiratory compromise. The mean follow-up was 16.4 years. Three patients were from an AD, 3 from an AR family and in 4 patients CMT was caused by de novo mutations. Respiratory compromise in the form of documented restrictive pulmonary disease was present in 5/10 (50 %) of the patients, in one case it resulted in respiratory failure and death at 6 years of age. Cranial nerve findings were present in 60 %, and involved facial nerve, cranial nerve III, IX and XII. The progression of the disease was rapid, moderate or mild, depending upon the various mutations. Interestingly the toxic gain of function mutations resulted in mild, moderate or severe progression depending on the mutation, while the homozygous loss of function mutation in the recessive family with three affected children had minimal if any progression of the neuropathy. We have performed in vitro functional studies, including transcriptional activity, localization and function in apoptosis in transient transfection experiments utilizing constructs generated by in vitro mutagenesis. We correlated the in vitro data with severity of disease as measured by age of onset and rate of progression. We did not find a correlation between mutations and outcome, thus it is extremely difficult to prognosticate patients with EGR2 mutations. However our study confirmed that respiratory compromise and cranial nerve dysfunction are commonly associated with EGR2 mutations and can be useful in guiding molecular diagnosis.

ANALYSIS OF FIVE GENES IN BRAZILIAN PATIENTS WITH NONSYNDROMIC MENTAL RETARDATION. *C.M.C. Maranduba, A. Vianna-Morgante, M.R. Passos-Bueno.* Centro de Estudos do Genoma Humano, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil.

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X-linked Mental Retardation affects approximately 1 in 600 males, comprising syndromic (MRXS) and nonsyndromic forms (MRX). To date 13 genes have been found to be associated with MRX. Four of these genes (*TM4SF2*, *IL1RAPL1*, *GDII*, *AGTR2*) and *ARHGEF9* gene, a functional candidate, were selected in this study. *TM4SF2* and *IL1RAPL1* mutations appear to account for 5-10% of MRX, while the other mutations are associated with less than 1% of the cases. We screened the coding region and the exon-intron boundaries of *TM4SF2*, *IL1RAPL1*, *GDII*, *AGTR2* and *ARHGEF9* genes, through SSCP/ dHPLC analysis and sequencing, in 118 male patients (25 familial and 93 isolated cases) who were shown not to carry the fragile X mutation. We found two polymorphisms in *TM4SF2* gene: c.237 CT (frequency of 3%) e c.441CT (5%), and a missense mutation c.515 CA (P172H), possibly pathogenic (Maranduba *et al.*, 2004). In the *IL1RAPL1* gene, we found one polymorphism (GA in 5UTR; 2% in our population) and an apparent silent mutation c.1200 GA (exon 9) not found in 221 control chromosomes. The analysis of this mutation in the RNAstructure 4.0 program (Nucleic Acids Res. 31:13, 3406-15, 2003) suggests that it changes the structure of the RNA and its possibly pathogenic. In order to further investigate if the mutation c.1200 GA in *IL1RAPL1* affects the processing of mRNA, we are currently conducting in vitro studies using the *pSPL3* vector (Exon trapping system, Invitrogen). We also identified the polymorphism IVS910GT in *GDII* gene and R248K in *AGTR2* gene, both already described. We did not find any pathogenic or polymorphic change in the *ARHGEF9* gene. In conclusion, our results suggest that the mutations P172H/*TM4SF2* and c.1200 GA/*IL1RAPL1* are possibly pathogenic, and that mutations in these genes account for approximately 1% of MRX cases. In this moment, *TM4SF2*, *IL1RAPL1*, *GDII*, *AGTR2* and *ARHGEF9* genes should not be considered in the routine diagnosis of mentally retarded individuals. Supported by: FAPESP/CEPID, CNPq.

Characterization of the mental retardation gene *FACL4* and analysis of a cellular Knock-out model. *I. Meloni¹, I. Longo¹, M. Bruttini¹, M.D. Ledesma², B. Hellias², C. Dotti², A. Renieri¹*. 1) Department of Molecular Biology, Medical Genetics, University of Siena, Siena, Italy; 2) Cavalieri Ottolenghi Scientific Foundation, University of Torino, Torino, Italy.

In 2002 we identified a new gene responsible for non-syndromic X-linked mental retardation (MRX): *FACL4* (fatty acid-CoA ligase 4), recently renamed *ACSL4*. The gene encodes for a protein which adds Coenzyme-A to long chain fatty acids, with high preference for arachidonic acid. It is expressed in all tissues except for liver and presents a brain-specific isoform resulting from alternative splicing and containing 41 additional N-terminal hydrophobic aminoacids. In order to establish how a mutation in this protein causes mental retardation we have characterized *ACSL4* expression in normal neuronal and non-neuronal cells. Western blot analysis was performed on protein isolated from brain, lymphoblasts and the neuronal cell line SH-SY5Y. This analysis demonstrated that in lymphoblasts *ACSL4* is present with two different isoforms. On the contrary, in brain and the neuronal cell line there is a third isoform with higher molecular weight, presumably corresponding to the brain-specific isoform. Immunofluorescence experiments in SH-SY5Y cells demonstrated that the protein is located in the medium compartment of Golgi apparatus. Analysis of cholesterol and ceramide content of membrane lipid extracts (total and from rafts) failed to demonstrate any difference between patients and control lymphoblasts. In order to define the possible role of *ACSL4* protein in neurons we have analyzed the consequences of its absence on neuronal differentiation in SH-SY5Y cells by employing antisense technology. This analysis has shown that differentiating cells lacking *ACSL4* expression present significantly longer cell processes with respect to cells expressing the protein, suggesting a role for *ACSL4* in membrane synthesis and/or recycling processes.

Program Nr: 2450 from the 2004 ASHG Annual Meeting

Mental retardation in Norrie disease due to vascular defects in the brain? *J. Neidhardt, U. Luhmann, B. Kloeckener-Gruissem, E. Glaus, W. Berger.* University of Zurich, Institute of Medical Genetics, Division of Medical Molecular Genetics and Gene Diagnostics, Schorenstrasse 16, 8603 Schwerzenbach, Switzerland.

Norrie disease (ND) is an X-linked recessive form of congenital blindness, progressive deafness and mental retardation. The disease is caused by mutations in the gene encoding Norrin, a ligand of the receptor Frizzled 4 of the Wnt signaling pathway. Norrie disease pseudoglioma homologue (*Ndph*) knockout mice closely resemble the human phenotype in eye and ear. They show defects in the vasculature of the inner retina and cochlea, while gross brain morphology is normal. In humans, at least one third of all ND patients show mental retardation with psychotic features, but its reasons are not yet understood. We studied the vasculature of different brain regions in our mouse model for ND, to elucidate a possible correlation between vascular defects in brain and mental retardation. The vascular network of the cerebellum, cortex, and hippocampus was stained by immunohistochemistry on cryosections of 8 month old mice and blood vessels were counted. We found a significantly reduced vascular content (~ 20 %) in the cerebellum of knockout mice. In addition, we observed an ~ 8 % decrease in the amount of blood vessels in both, cortex and hippocampus. These results were correlated with the transcription level of *Ndph* in different brain regions, examined by quantitative RT-PCR. We found that *Ndph* is expressed three times higher in the cerebellum than in cortex and hippocampus. Our results suggest a dosage dependent correlation between transcriptional activity of the *Ndph* gene and the amount of blood vessels in different brain regions. These data provide a first hint towards a possible cause of mental retardation in Norrie disease patients.

Program Nr: 2451 from the 2004 ASHG Annual Meeting

Identification of genetic basis in Korean patients with Charcot-Marie-Tooth disease type 1 (CMT1). *H-K. Park, C-S. Ki, E-J. Kim, J-W. Kim.* Department of Laboratory Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea.

Charcot-Marie-Tooth disease type 1 (CMT1), the most common hereditary neurological disorder in humans, is characterized by clinical and genetic heterogeneity. About 70~80% of CMT1 patients have been associated with the duplication of 1.5 Mb region on chromosome 17p11.2-p12 including the PMP22 gene (PMP22-DUP), while remaining patients have been due to mutations in the PMP22, MPZ, GJB1, EGR2, and LITAF genes. In the present study, we tried to reveal the genetic basis of Korean patients with CMT1. Thirty-six index cases with CMT1 were included and tested for the PMP22-DUP by the pulsed-field gene electrophoresis (PFGE)-Southern blot analysis. Patients without PMP22-DUP were further investigated for the presence of point mutations in PMP22, MPZ, GJB1, EGR2, and LITAF genes by direct sequencing of all coding exons and their flanking intronic regions. Twenty-seven patients (75%) were confirmed to have the PMP22-DUP by the PFGE-Southern blot analysis, which was concordant with the previous reports. Among the patients with PMP22-DUP, 6 (17%) patients had point mutations: two in the PMP22 gene (T23R and 179-2A_G) and four in the GJB1 gene (D46G, V95M, Y151H, and F180L), in which three mutations were novel. No mutation was found in the MPZ, EGR2, and LITAF genes. In summary, the PMP22-DUP is also predominant in Korean patients with CMT1. It is of note that there was no patients with MPZ, EGR2, and LITAF mutation, which have been associated with about 25% of CMT1 patients.

Autosomal dominant early-onset Alzheimer's disease: an update. G. Raux¹, L. Maréchal^{2, 3}, C. Martin², J. Bou¹, C. Penet⁴, A. Brice⁴, D. Hannequin^{1, 3}, T. Frebourg^{1, 2}, D. Campion¹. 1) Inserm U614, Faculty of Medicine, 76183 Rouen, France; 2) Department of Genetics, Rouen University Hospital, 76031 France; 3) Department of Neurology, Rouen University Hospital, 76031 France; 4) Inserm U589 la Salpêtrière Hospital 75013, Paris, France.

Autosomal dominant early-onset Alzheimer's disease (ADEOAD) is a rare condition which is genetically heterogeneous. Missense mutations in three genes, encoding for the presenilin 1 (*PSEN1*), presenilin 2 (*PSEN2*) and amyloid precursor protein (*APP*) have been identified in ADEOAD. Mutational analysis of these 3 genes in 34 families had previously allowed us to estimate that 71 % of ADEOAD families were attributable to mutations of *PSEN1* and *APP* genes. However, other groups have recently reported lower estimates on smaller samples. To further clarify the respective contribution of these genes to ADEOAD, we analyzed 25 novel families ascertained by using the stringent criteria that we had previously defined (i. e. the occurrence of probable or definite AD cases with onset before age 60 in three generations). We identified *PSEN1* mutations (including 7 previously unreported mutations) in 19 families and *APP* mutations in 4 families. We failed to detect any *PSEN2* mutation. The new mutations cosegregated with the disease in two families with multiple living affected members. Two *PSEN1* mutations (Pro264Leu and Phe386Ser) were associated with an atypical presentation including spastic paraparesis. The Glu318Gly variation, which has been described either as a non pathogenic polymorphism or as a mutation with reduced penetrance was present in three families. Finally, 78 % of the affected subjects had an *APOE* 3-3 genotype and none had the *APOE* 4-4 genotype. When these data are combined with those previously published, and taking into account that the diagnosis of AD was not confirmed by neuropathological examination in two previously negative families we conclude that among 59 ADEOAD families, 65% are attributable to *PSEN1* mutations (59% if the Glu318Gly variation is considered as a non pathogenic polymorphism) and 15 % to *APP* mutations while 20% of the ADEOAD remain unexplained.

Mutations in the DLG3 gene cause non-syndromic X-linked mental retardation. *F.L. Raymond¹, P. Tarpey², J. Parnau¹, M. Blow², H. Woffendin², J. Moon¹, M. Partington⁴, G. Turner⁴, R. Stevenson⁵, C. Schwartz⁵, I. Young⁶, M. Bobrow¹, P.A. Futreal², M.R. Stratton², J. Gecz³, R. Wooster², on behalf of the Genetics of Learning Disability Study.* 1) Dept Medical Genetics, CIMR, Cambridge, United Kingdom; 2) Sanger Institute, Hinxton, Cambridge UK; 3) Womens and Childrens Hospital and University of Adelaide, Adelaide, Australia SA 5006; 4) Hunter Genetics and University of Newcastle, PO Box 84, Waratah, New South Wales, Australia 2298; 5) Greenwood Genetics Centre, 1 Gregor Mendel Circle, Greenwood, South Carolina USA 29646; 6) Department of Medical Genetics, Leicester Royal Infirmary, Leicester LE1 5WW, UK.

We have identified truncating mutations in the human gene DLG3 the homolog of Drosophila discs large 3 gene in 4 out of 329 families with moderate to severe X-linked mental retardation. DLG3 encodes synapse-associated protein 102 (SAP102), a member of the membrane associated guanylate kinase (MAGUK) protein family. Neuronal SAP102 is expressed during early brain development and is localised to the post-synaptic density of excitatory synapses. It is composed of 3 amino terminal PDZ domains, a src homology (SH3) domain and a carboxyl terminal guanylate kinase (GK) domain. The PDZ domains interact directly with the NR2 sub-units of the NMDA glutamate receptor, and with other proteins responsible for NMDA receptor localization, immobilization and signaling. The mutations identified in this study all introduce premature stop codons within, or before the third PDZ domain, and it is likely that this impairs the ability of SAP102 to interact with the NMDA receptor and/or other proteins involved in downstream NMDA receptor signaling pathways. The disruption of NMDA receptor targeting or signaling due to loss of SAP102, may lead to altered synaptic plasticity, and offers a mechanism which may explain the intellectual impairment observed in DLG3-mutated individuals.

Identification of a more common founder mutation in the *senataxin* gene responsible for AOA2 in French-Canadians. K. Roddier¹, A. Duquette¹, J. Mc Nabb-Baltar¹, I. Gosselin¹, A. Saint-Denis¹, M.J. Dicaire¹, L. Loisel¹, L. Marchand², J. Mathieu³, J.P. Bouchard⁴, B. Brais^{1,2,3}. 1) Laboratoire de neurogénétique, Centre de recherche du CHUM, Université de Montréal, Montreal, Quebec, Canada;; 2) Clinique d'ataxie du CHUM, Centre Hospitalier de l'Université de Montréal, Montreal, Quebec, Canada; 3) Clinique des maladies neuromusculaires, Carrefour de la Santé de Jonquière, Saguenay, Quebec, Canada; 4) Service de neurologie de l'Hôpital de l'Enfant-Jésus, Université Laval, Quebec, Quebec, Canada.

Recessive ataxias are a heterogeneous group of neurodegenerative diseases. At least one form of ataxia is more frequent in the Quebec population: the autosomal recessive spastic ataxia of Charlevoix-Saguenay (MIM 270550). In 1980, Jean-Pierre Bouchard described seven members of a large family who presented a rare recessive form of ataxia (MIM 606002). In this study, we further characterized this family and described 17 additional cases from nine families. The gene *senataxin* responsible for this disease has recently been identified. Following informed consent, we extracted DNA of 23 patients and their relatives. We completed a linkage analysis using standard methods. Further to the identification of the responsible gene, we sequenced all the exons and the intron/exon junctions in order to search for mutations in the French-Canadian cohort. Linkage analysis has demonstrated that all families are linked to the 9q34 locus for the recessive Ataxia with Ocular Apraxia type 2 (AOA2, MIM 606002). A maximum LOD score of 9.86 was obtained at = 0 for marker D9S2157. We sequenced the *senataxin* gene responsible for the AOA2 and identified four mutations in the French-Canadian population, including two novel missense mutations. The most common founder mutation is shared by 85% of the carrier chromosomes. AOA2 becomes the second recessive ataxia to be identified to be more frequent in the French-Canadian population due to the higher prevalence of a founder mutation.

***SIMPLE/LITAF* mutations in Charcot-Marie-Tooth disease and the potential role of the protein products in peripheral nerve transcriptional regulation and protein degradation.** *G.M. Saifi*¹, *K. Szigeti*¹, *W. Wiszniewski*¹, *M.E. Shy*², *K. Krajewski*², *I. Hausmanowa-Petrusewicz*³, *A. Kochanski*³, *S. Reeser*⁴, *J.R. Lupski*^{1,5,6}. 1) Dept Molec & Human Genetics, Baylor College of Medicine, Houston 77030, TX; 2) Dept Neurology, Wayne State University, Detroit, MI; 3) Neuromuscular Unit, Medical Research Centre, Polish Academy of sciences, Warsaw, Poland; 4) WellSpan Health System, York Hospital, York, PA 17505; 5) Dept Pediatrics, Baylor College of Medicine, Houston, TX; 6) Texas Childrens Hospital, Houston TX.

Charcot-Marie-Tooth (CMT) disease is a clinically and genetically heterogeneous group of inherited peripheral neuropathies characterized by progressive weakness and atrophy of distal limb muscles. Recently, a transcription factor gene, *SIMPLE/LITAF*, was shown to be responsible for an autosomal dominant demyelinating form of CMT linked to 16p (CMT1C). We screened this gene for mutations in a cohort of 192 patients with CMT or related neuropathies, each of whom tested negative for other known genetic causes of CMT. In 16 unrelated CMT families we identified nine different nucleotide variations in *SIMPLE/LITAF* that were not detected in control chromosomes. *SIMPLE/LITAF* mutations can occur *de novo* associated with sporadic CMT1. Bioinformatics analyses of LITAF and its cognate recognition sequence suggest involvement in global peripheral nerve transcription. We propose that a WW domain containing protein, most likely NEDD4, activates LITAF by the regulated ubiquitin/proteasome-dependent processing (RUP) pathway. Bioinformatics analyses and other observations of SIMPLE, an alternatively spliced isoform of LITAF, suggest that it could be a member of the RING finger motif-containing subfamily of E3 ubiquitin ligases that are associated with the ubiquitin-mediated proteasome processing pathway. It is thus likely that the transcriptional regulation by LITAF or the E3 ubiquitin ligase activity of SIMPLE may underlie the pathogenesis of Charcot-Marie-Tooth disease.

Hereditary Spastic Paraplegia 3A: a novel Atlastin-1 mutation causes aggregate formation in mammalian cells.

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Hereditary Spastic Paraplegia is a heterogeneous group of disorders characterized by progressive lower extremity weakness and spasticity. Thus far, twenty-two SPG loci have been mapped and ten genes identified. Recently, autosomal dominant mutations in the Atlastin-1 gene have been found to be associated with SPG3A which account for 10% of all known SPGs. Atlastin-1 is a 64 kDa member of the dynamin/Mx/guanylate-binding protein superfamily of large GTP-ases having two predicted transmembrane domains. Based on sequence homology and cellular localization, a functional involvement in Golgi membrane dynamics and vesicle trafficking have been proposed for Atlastin-1. We analyzed a five-generation Mongolian family in which SPG3A segregates with a novel mutation Thr156Ile in the Atlastin-1 gene. Four family members were diagnosed with definite SPG and had severe spastic gait and lower extremities hyperreflexia. The oldest affected member, a 47 year old female, had in addition, cognitive problems, severe bilateral weakness in the lower and distal muscles of the upper extremities, brisk reflexes, markedly increased muscle tone, up-going plantars, pes cavus. Other patients had hand tremor, urinary urgency and incontinence, and distal hypesthesia for pain and touch. Evidence is presented that mutant Atlastin-1 leads to formation of aggregates in the mammalian cells. Formation of aggregates may occur due to protein misfolding and disruption of oligomerization of Atlastin-1, which in turn may have deleterious effects on neuron survival. Immunoblots and immunocytochemical studies revealed that Atlastin-1 is associated with alpha-tubulin and endoplasmic reticulum (ER).

Respiratory and bioaminergic alterations in *Mecp2*-deficient adult mice, an experimental model for Rett

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Rett syndrome is a severe X-linked neurological disorder, in which most patients carry a mutation in the gene encoding methyl-CpG binding protein 2 (MECP2). The clinical course of the disease consists of normal in utero and neonatal development followed by a period of regression showing signs of neurodevelopmental defects (arrest of brain development, loss of acquisitions such as speech and walk, apparition of behavioural troubles). One of the most life-threatening symptoms is the presence of severe breathing alterations consisting of a combination of hyperventilation and apneas when the patient is awake. These alterations disappear when the patient is sleeping. Several hypothesis have been proposed to explain these observations such as a defect in autonomic functions, brainstem immaturity and/or altered neurotransmitter systems. Studies of bioaminergic levels have been performed but revealed inconsistent results : normal concentration of noradrenaline, serotonin and dopamine in the spinal fluid whereas brain autopsy showed reduced levels of these substance. We performed experiments on wild-type and *Mecp2*-deficient mice to understand the role of the *Mecp2* gene in respiration and bioaminergic systems. Using plethysmography to record breathing, high pressure liquid chromatography to measure the concentration of bioamines and immunolabelling to study the catecholaminergic neurons, we show that the deficiency in the *Mecp2* gene lead to an altered respiratory rhythm and an abnormal medullary bioaminergic content.

Analysis of the GABA-A receptor gene in Japanese patients with Acute encephalitis or encephalopathy with refractory, repetitive partial seizures. *N. Shiroma, H. Minema, K. Katsuren, S. Fukuyama, T. Ohta.* Pediatrics, University of the Ryukyus, Okinawa, Japan.

<Background> Acute encephalitis or encephalopathy with refractory, repetitive partial seizures (AERRPS) is a rare disease caused by unknown etiology. In some case, overdose benzodiazepine which acts on GABA-A receptor, is effective to control of seizure. Recent studies conformed that some mutations in GABA-A receptor alpha1 subunit (GABRA1) gene alter the functional properties of GABA-A receptors. <Objective> To evaluate the genetic contribution of cloned human GABA-A receptors to AERRPS. <Methods> Informed consent was obtained from all the patients in this study. We analyzed GABRA1 gene in 6 patients with AERRPS. <Results> We found no mutation in the exons of GABRA1, we did identify two single nucleotide polymorphisms (SNPs) in exon 4 (471 C > T) and exon 6 (679 C > T). We postulate that the GABRA1 gene might not be a susceptibility gene for AERRPS.

Neuronal inclusions are not pathogenic in HD: a YAC mouse expressing a truncated fragment of huntingtin (shortstop) with 128 CAGs displays widespread neuronal inclusions but no behavioural changes or neurodegeneration. *E. Slow*¹, *R. Graham*¹, *A. Osmand*², *R. Wetzel*², *R. Devon*¹, *G. Lu*¹, *Y. Deng*¹, *J. Pearson*¹, *K. Vaid*¹, *N. Bissada*¹, *B. Leavitt*¹, *M.R. Hayden*¹. 1) Medical Genetics, CMMT, University of British Columbia, Vancouver, BC Canada; 2) Graduate School of Medicine, University of Tennessee, Knoxville, TN.

A YAC mouse model expressing full-length human huntingtin (htt) with 128 CAGs (YAC128) under the control of 24 kb of upstream promoter, exhibits behavioural and neuropathological changes that accurately recapitulate Huntington disease. During the development of other full-length YAC128s, a mouse expressing a short fragment of htt (shortstop) was serendipitously created. The shortstop mouse which expresses exons 1 and 2 of the HD gene, producing a protein of 117 amino acids with an expanded polyglutamine tract, possesses the identical regulatory region, polyglutamine repeat and level of transgenic protein as the full-length YAC128 on the identical FVB strain. This has allowed for the first time the opportunity to compare and contrast the effects of both full-length and truncated mutant htt *in vivo*. Htt inclusion analysis in the shortstop mice revealed 6-9 month earlier onset and more widespread inclusion formation both in percentage of neurons (65-95% more in 18 month shortstop compared to age-matched YAC128s) and in neuronal tissue types affected in the shortstop compared to the full-length YAC128. In addition, aggregation foci, a precursor of htt inclusions, are present at a higher density in certain tissues of the shortstop compared to the YAC128. The YAC128 model demonstrates reproducible decreases in rotarod performance ($p < 0.001$), brain weight ($p < 0.01$), striatal volume ($p < 0.05$) and striatal neuronal count ($p < 0.05$) compared to wild-type littermates. Surprisingly, shortstop does not demonstrate deficits in any of these measures, despite widespread inclusion burden. The role of inclusions in HD pathogenesis is controversial. These results indicate that htt inclusions and precursors, although part of the disease sequelae, are not pathogenic, and can exist completely separate from HD-related neuronal dysfunction and degeneration.

Aberrant striatal phosphatidylserine decarboxylase gene expression in the HPRT-knockout mouse. *S. Song, T. Friedmann, D. Smith.* Pediatrics, Univ. Calif. San Diego, La Jolla, CA.

There is virtually no detailed understanding of the mechanisms responsible for the dopamine deficit in the basal ganglia of the HPRT-knockout mouse, a model of Lesch Nyhan disease. We have used microarray transcriptional profiling methods in wild type and HPRT-knockout mice to examine the possibility that secondary genetic aberrations play a role in the altered CNS function in this disease model. We have identified a small number of genes whose expression in the striatum is aberrant during the development of the dopamine deficient from 2-6 weeks of post-natal life. One of the principal differentially expressed genes is the gene encoding phosphatidylserine decarboxylase (PSD) which, together with several other members of the PSD-like family, is significantly down-regulated in the striatum of the HPRT-knockout mice. The microarray results were confirmed by quantitative PCR analysis and by in-situ hybridization. Specifically, these results suggest suggest a potential role for altered phospholipid metabolism in the development of the CNS phenotype in the Lesch Nyhan disease. More generally our results suggest that complex gene expression defects may be responsible for parts of the phenotype of even "monogenic" and that at least some classical monogenic disorders may be functionally multigenic.

Modeling of spinocerebellar ataxia 7 in *Drosophila melanogaster* as a model of polyglutamine diseases. G.

Stevanin^{1,3}, *M. Latouche*¹, *E. Martin*¹, *V. Monnier*², *A. Mouatt-Prigent*¹, *A. Brice*^{1,3}, *H. Tricoire*². 1) Inst des Neurosciences, INSERM U289-NEB, Paris, France; 2) Institut Jacques Monod, UMR7592 CNRS, Paris, France; 3) Department of Genetics, Cytogenetics and Embryology APHP, Hopital Salpetriere, Paris, France.

Spinocerebellar ataxia type 7 (SCA7) is one of the 6 autosomal dominant cerebellar ataxias (SCA1, 2, 3, 6, 7 and 17) belonging to the group of the polyglutamine (poly-Q) diseases, which also includes Huntingtons disease, spinobulbar muscular atrophy and dentatorubral-pallidoluysian atrophy. Poly-Q diseases are all neurodegenerative disorders caused by the expansion of a translated (CAG)_n repeat in the corresponding gene and they share several features including the presence of neuronal intranuclear inclusions (NIIs) in patients brains. The mechanisms of neuronal death and the role of the NIIs remain unclear. In order to identify the pathways involved in the physiopathological process, we generated SCA7 transgenic flies expressing a truncated form of wild type or expanded Ataxin7 (polyQ-Ataxin7), the protein encoded by the SCA7 gene. Several transgenic lines were established and expression of the transgenes was targeted to the nervous system, muscles or ubiquitously, and was analyzed by western blot, real time RT-PCR, immunofluorescence, immunohistochemistry and electron microscopy. Preliminary results indicate that expanded Ataxin7 aggregates within the nucleus and induces late-onset lethality, early adult death, reduced viability and larval lethality according to the targeted tissues. Based on these results, we will use this model to identify modifier genes and pharmacological compounds that might prevent or modulate the toxicity of polyQ-Ataxin7.

Evaluation of Parkin mutations in a large familial PD cohort: The GenePD Study. *M. Sun¹, G. Xu², J. Latourelle², R. Prakash², J. Maher², R.H. Myers², J.F. Gusella¹, for the GenePD Study.* 1) Molecular Neurogenetics Unit, Center for Human Genetic Research, Massachusetts General Hospital, Harvard Medical School Boston, MA; 2) Department of Neurology, Boston University School of Medicine, Boston, MA.

Mutations in the Parkin gene are the most commonly identified cause of young onset parkinsonism with autosomal recessive inheritance. The frequency and spectrum of Parkin mutations have been extensively studied in ethnically mixed patient populations. Overall, the frequency of Parkin mutations was estimated at up to 50 percent in ARJP, 10-25 percent in early onset, and 2 percent in late onset families. However, the mutation rate reported varied by PD samples tested and detection methods employed. The purpose of this study was to evaluate the burden of Parkin mutations in 329 families with at least two PD affected members collected in the GenePD Study. We comprehensively screened all 12 coding exons and the corresponding exon-intron boundaries by both conventional mutation screening and gene dosage analysis. We narrowed the focus of our screen to the subset of families most likely to have Parkin mutations. We genotyped the microsatellite marker D6S305 which lies within the intron7 of the parkin gene in our entire sample set containing 715 individuals from 329 families. The 83 families where affected siblings shared both allelic IBS at the parkin locus plus an additional 99 families containing at least one member with onset age earlier than 52 years. In total, we screened 182 index patients (selecting the youngest onset subject in each family). We performed SSCP and sequencing to detect point mutations in the index patients combined with quantitative duplex PCR using Lightcycler to detect alterations of gene dosage (exon deletions and duplications). Mutations were found in 29/182 patients. Seven were compound heterozygous; 2 were homozygous; and 20 were heterozygous. We identified 5 known point mutations in 7 patients and 9 gene dosage alterations in 25 patients. In conclusion, Parkin mutations are not rare in this sample of PD patients (16%) and screening reduces genetic heterogeneity in the study of familial PD.

DJ-1 gene in late-onset recessive Parkinsons disease. *P. Tarantino, F. Annesi, D. Civitelli, E.V. De Marco, I.C. Ciro' Candiano, S. Carrideo, F.E. Rocca, G. De Cicco, G. Annesi.* Inst of Neurological Sciences, National Research Council, Mangone, Cosenza, Italy.

Three loci have been associated with early-onset, autosomal recessive parkinsonism: Parkin (PARK 2,) DJ-1 (PARK 7), PINK1 (PARK 6). Recently, mutations in DJ-1 were described as a novel cause of early-onset parkinsonism. We analysed the DJ-1 gene in 23 subjects with late onset (after the age of 45 years) Parkinsons disease (PD) screened from 18 families originating from Southern Italy with autosomal recessive PD (LORPD). These patients were evaluated previously for the presence of parkin mutations (PARK2) by a combination of gene dosage and sequencing and were found to be negative. The 18 families with LORPD were selected according to the following criteria: reported parkinsonism in two or more siblings; a mode of inheritance compatible with autosomal recessive transmission (affected siblings without affected parents); an age at onset of 45 years or older in all the affected siblings. Genomic DNA was extracted in all subjects from peripheral-blood leukocytes according to standard procedure. The entire DJ-1 open reading frame was amplified from genomic DNA, and PCR amplified were directly sequenced by means of forward primers. We did not find any pathogenic mutation in the DJ-1 gene in all patients with LORPD. We identified at codon 98 (exon 5) a G/A heterozygous substitution in four patients; this change was confirmed by Msp I restriction analysis, and was found in 10 out of 700 control chromosomes. Moreover we identified an 18 bp insertion/deletion variant in the promoter region of DJ-1(g.168_185del) in six patients; this variant was also found in 3 out of 600 control chromosomes. Our results indicate that mutations in DJ-1 are not common cause of LORPD. Further studies by using quantitative PCR assay are needed to exclude heterozygous exons rearrangements in subjects with LORPD.

An unusual cosegregation of CMT1A duplication and HNPP *PMP22* point mutation in a family with CMT1 disease. *F. Taroni, M. Milani, D. Pareyson, D. DiBella.* Div. of Biochemistry & Genetics, Ist. Naz. Neurologico C. Besta, Milan, Italy.

The common CMT1A duplication and HNPP deletion arise from unequal crossing over and homologous recombination during meiosis between flanking repeats (CMT1A-REP). CMT1A and HNPP thus result from an altered copy number of the dosage-sensitive myelin gene *PMP22* which is included in the duplicated/deleted tract. However, both diseases can also be caused by rare *PMP22* point mutations. We report here a unique family carrying both the CMT1A duplication and a HNPP *PMP22* point mutation. A CMT1 child (III-1), the only affected member of her family, was found to carry the duplication. Surprisingly, the mutation was also found in the mother (II-2) who had normal clinical and electrophysiologic phenotype. *PMP22* sequence analysis in both individuals uncovered a truncating frameshift mutation (Leu145fs) in the healthy mother but not in the affected child. In the mother, this mutation, which had been previously identified by us in 3 other HNPP families, would inactivate the extra copy of the *PMP22* gene, functionally compensating the effects of the duplication. Further investigations in the family demonstrated that the healthy maternal grandmother (I-1) also carried both the duplication and the point mutation, while her other son (II-1) had neither of them, which indicates that both mutations were harbored by the same chromosome. Microsatellite analysis suggests that a meiotic intrachromosomal homologous recombination event of maternal origin has occurred in the affected child, replacing the mutated *PMP22* copy with a functional one and resulting in CMT1 phenotype. [Supported by grants from Ministero della Salute (RF 2002/160) and Fondazione Mariani to F.T.]

Toward a clinical and molecular genetic description of a new form of autosomal recessive spastic ataxia in the French-Canadian population. *I. Thiffault^{1,2}, M.F. Rioux¹, J. Poirier¹, J. Mathieu³, G. Rouleau⁴, J.P. Bouchard⁵, B. Brais^{1,2}.* 1) Dept of Neurogenetics, Room M411L, CHUM-Notre-Dame, Montreal, PQ, CANADA; 2) Dept of Molecular Biology, Université de Montreal, PQ, CANADA; 3) Carrefour de la santé de Jonquière, Jonquière, PQ, CANADA; 4) MUHC-Montreal General hospital, Montreal, PQ, CANADA; 5) CHUL, Quebec, PQ, CANADA.

Recessive spastic ataxia are a very heterogeneous group of neurodegenerative disorders characterized by symptoms and signs of progressive gait, ataxia, spasticity, peripheral neuropathy, pyramidal and/or extrapyramidal signs and in certain cases cardiomyopathy, hypermyelinated retinal nerve fibers, and dysarthria. Relying on a network of Ataxia and Neuromuscular clinics that cater to more than 500 ataxic patients, we have recruited 26 affected individuals and their unaffected relatives from 20 unrelated French-Canadian families with an original form of autosomal recessive ataxia which do not have a precise molecular diagnosis; all patients were negative for Friedreich ataxia and Spastic ataxia of Charlevoix-Saguenay (SACS) by linkage analysis. Neuroimaging (MRI) shows cerebellar atrophy in 100% of the cases; more severe cases presented mild to moderate cerebral atrophy (42.9% of the cases) and leukodystrophy (52.4% of the cases). The major clinical features are the presence of ataxia, dysarthria, spasticity and hyperreflexia. The age of onset is variable (mean 8 years; range 3-20). The more severe cases have scoliosis, dystonia and mild cognitive impairment. There is a great intrafamilial and interfamilial variability in the severity of the phenotype. The clinical phenotype of the families is consistent with an autosomal recessive form of inheritance and geographic distribution of the cases suggests a French-Canadian founder effect. Using a homozygosity mapping approach, we performed a systematic 10cM genome wide scan (GWS) which did not reveal the responsible locus for this recessive ataxia. However, microsatellite analysis is on-going to refine the GWS map and to exclude 8 different known loci of other disorders which could overlap with this new form of autosomal recessive spastic ataxia. This study could lead to the identification of the mutated gene responsible for approximately 20% of recessive ataxia in Quebec and possibly in other populations with founder effect.

Refinement of the locus for pyridoxine-dependent epilepsy on chromosome 5q31. *B. Uhlenberg¹, P. Koehne², J. Herrmann², B. Lucke¹, M. Schuelke¹, B. Weschke¹.* 1) Department of Neuropediatrics, Charite, Berlin, Germany; 2) Department of Neonatology, Charite, Berlin, Germany.

Pyridoxine-dependent epilepsy (MIM 266100) is an autosomal recessive disorder characterized by different types of convulsions starting in the neonatal period, infancy or even in utero. Seizures exclusively respond to immediate administration of pyridoxine hydrochloride (vitamin B6). Interruption of pyridoxine supplementation usually leads to the recurrence of seizures which makes pyridoxine dependence permanent. Hypo- or dystonia, jitteriness, a high-pitched cry, abdominal distension, frequent vomiting, hypothermia as well as moderate motor and mental retardation are possible associated symptoms. The pathogenesis of pyridoxine-dependent seizures is not known, but a reduction in the synthesis of gamma-aminobutyric acid (GABA) as a result of diminished activity of the glutamic acid decarboxylase (GAD) has been postulated. However, linkage to the GAD1 (2q31) and GAD2 genes (10p23) was previously excluded in 4 consanguineous families and genome-wide scanning revealed linkage of the underlying gene to markers on chromosome 5q31.2-q31.3. In the absence of an appropriate candidate gene the responsible gene defect has not been unraveled so far. In this study we investigated a consanguineous Turkish family with two affected sons with unequivocal symptoms of pyridoxine-dependent epilepsy. We performed homozygosity mapping by microsatellite markers and confirmed linkage to the 5q region. We narrowed the critical region by detection of a recombination event proximal to marker D5S2033. The two patients were homozygous at loci D5S658 to D5S436. Thus the region encompassing the disease-underlying gene now comprises 4.5 cM. Work is in progress in order to identify a mutation in a possibly disease-related gene.

Search for rearrangements at the Golli-MBP locus in patients with undetermined leukodystrophies (UL) using different genomic quantification techniques. *C. Vaurs-Barriere¹, MN. Bonnet-Dupeyron^{1, 2}, P. Combes¹, F. Gauthier-Barichard¹, P. Vago², O. Boespflug-Tanguy¹.* 1) UMR384, Medicine Faculty, Clermont-Fd, France; 2) Cytogenetics Laboratory, Medicine Faculty, Clermont-Fd, France.

Leukodystrophies represent a heterogeneous group of rare inherited disorders that involve primarily the white matter of the central nervous system (CNS). The PLP gene is implicated in the etiology of the hypomyelinating X-linked forms of leukodystrophies; large rearrangements of the PLP genomic region being the predominant mutation. Nevertheless, we found that almost half of the patients with a hypomyelinating leukodystrophy have no identified PLP mutation, suggesting that other genes important for brain myelination may be implicated. The myelin basic proteins (MBP) correspond to the second most important class of CNS myelin proteins. The MBP gene, localized on chromosome 18, is included in a more complex gene structure called the Golli-MBP complex. Rearrangements within the MBP gene have been found in spontaneous dysmyelinating mutants as the shiverer mouse and the long evans rat. Patients with 18q- syndrome displayed also abnormal myelination pattern on MRI correlated with a genomic minimal interval including the MBP gene. Therefore, the Golli-MBP locus could be considered as a good candidate gene in the etiology of UL. We first searched for rearrangements at the Golli-MBP locus in 340 patients without identified PLP mutations. After using FISH in a pre-screening step, we have adapted the Multiplex Amplifiable Probe Hybridization (MAPH) and the Quantitative Mutiplex PCR of Short Fluorescent fragments (QMPSF) quantification techniques to the study of the Golli-MBP gene in our large cohort. Although preliminary results obtained by FISH led us to think that there may be duplication events in a mosaic form, no rearrangement has been finally found by QMPSF and MAPH, neither by FISH using a different labeling protocol. This work highlights the difficulty to use FISH for rearrangement detection (particularly duplications) and has allowed us to compare advantages and disadvantages between QMPSF and MAPH. Finally, rearrangements at the Golli-MBP locus seem not involved in the etiology of UL.

SCA8 EXPANSIONS ARE NOT FREQUENTLY ASSOCIATED WITH OTHER SCA EXPANSIONS IN SPANISH SERIES. *V. Volpini¹, J. Corral¹, I. Banchs¹, J. Armstrong^{1, 2}, H. Nicolas¹.* 1) Medical and Molecular Genetics Centre, Cancer Research Institute, Hospitalet de LL, Barcelona, Spain; 2) Neuropathologic Institut, Bellvitge Hospital, Barcelona, Spain.

The spinocerebellar ataxias (SCAs) belong to a group of genetic disorders caused by repeat expansion of a specific tandem DNA repeat. Several SCA loci cause dominantly inherited ataxia by the expansion of a CAG repeat which is translated into polyglutamine tracts. In contrast, SCA 8 is reported to be caused by an unstable transcribed but untranslated CTG repeat expansion in the 3 exonic region of a gene on chromosome 13q21, and large alleles above the disease threshold have been observed in healthy individuals who have no family history of ataxia. Additionally, the coexistence of SCA8 expansions with other expansions such as FRDA, SCA6 or SCA1 have also been reported elsewhere. We have studied a total of N = 203 patients with ataxia from families with a dominant pattern of inheritance and in which we have detected their respective SCA expansions: 38 SCA1; 47 SCA2; 91 SCA3; 5 SCA6 and 22 SCA7. We performed a PCR analysis in all cases using 5-fluorescent SCA8-F3 and SCA8-R4 primers as described and none of them also have the SCA8 expansions in the reported pathogenetic range. We have not found either great or giant expansions as reported in healthy individuals in other SCA series. In conclusion we have not found coexistence of SCA8 expansions with other SCA expansions in our SCA series. Such a situation seems to be a rare event although it has been detected in other SCA or FRDA series but it should be taken in account to explain the etiopathogenesis of dynamic mutations disorders.

Clinical and molecular genetic study of six Japanese families with familial hemiplegic migraine/ episodic ataxia type 2. T. Wada¹, T. Go², H. Tsukahara³, M. Kanayama³, Y. Suzuki⁴, Y. Fukushima¹, S. Saitoh⁵. 1) Medical Genetics, Shinshu Univ Sch Medicine, Matsumoto, Nagano, Japan; 2) Tokyo Women's Medical College, Tokyo, Japan; 3) Nagoya City Medical College, Nagoya, Japan; 4) Aichi Welfare Center for Persons with Developmental Disabilities, Kasugai, Japan; 5) Hokkaido Univ. Sch. Med., Sapporo, Japan.

Introduction Familial hemiplegic migraine (FHM; MIM#131500), episodic ataxia type 2 (EA2; MIM#108500) and spinocerebellar ataxia type 6 (SCA6; MIM#183086) are allelic disorders caused by mutations of the *CACNA1A* gene at 19p13. *CACNA1A* encodes a pore-forming Cav2.1 subunit of a P/Q-type voltage-dependent calcium channel. We reported here clinical and molecular genetic study of six Japanese families in which patients were clinically diagnosed as FHM or EA2. **Subjects** Patients from two FHM and four EA2 families, and 50 normal controls. **Methods** Genomic DNAs extracted from peripheral blood were amplified by PCR using primers designed for 47 exons of the *CACNA1A* gene, and the entire coding region including exon-intron boundaries was sequenced. Mutations were confirmed by DHPLC and/or PCR-RFLP. **Results** Two nucleotide substitutions (Thr666Met and Arg1057Cys) in two FHM families, and three nucleotide substitutions (Ala236Pro, Gly1105Ser and Gly1479Val) and one nonsense mutation (Arg1665stop) in four EA2 families were identified. **Discussion** We concluded that Thr666Met and Arg1665stop were pathogenic mutations. Arg1057Cys and Gly1105Ser were considered to be polymorphisms, because the polymorphisms did not cosegregate with affected family members, Therefore, the *CACNA1A* gene was not linked to the EA2 phenotypes in these families, suggesting that EA2 could be genetically heterogeneous, and some genes other than the *CACNA1A* gene might be involved in EA2. We have not decided yet whether Ala236Pro or Gly1479Val are pathogenic. Because FHM/EA2 has genetic heterogeneity and wide clinical spectrum in each family, comprehensive genetic analysis including functional study is mandatory to determine the pathological significance of the nucleotide substitutions. Our results should contribute to establishment of genotype and phenotype relationship in FHM/EA2.

Are the 5-hydroxytryptamine (5-HT) Receptor Genes HTR2B and HTR2C (Serotonin Receptor 2B and 2C) Modifier Genes for the Neurological Phenotype in Tuberous Sclerosis Complex? *C.H. Ward, H. Northrup, A.T. Williams, K.-S. Au.* Pediatrics, Univ of Tx at Hou, Houston, TX.

Tuberous sclerosis complex (TSC) is an autosomal dominant neurocutaneous disorder involving benign growths in the skin, brain, heart and kidneys of affected patients. The neurological phenotype of these patients is variable even within families and may be severe with early onset of seizures and significant mental retardation. We hypothesize that the action of modifier genes influences the phenotype of patients with mutations in the TSC1 and TSC2 genes. The 5-hydroxytryptamine (5-HT) receptors are a family of receptors for the neurotransmitter serotonin that act via stimulation of phospholipase C to produce a variety of central and peripheral effects. The HTR2B receptor is primarily involved in vasoconstriction, and possibly associated with pulmonary hypertension. HTR2C has been implicated in control of neuroendocrine function, body temperature and food intake. Polymorphic variants of the HTR2B and HTR2C have been tested for possible roles in a variety of psychiatric disorders, obesity, and neuronal hyper-excitability and seizure activity. The variant in HTR2B involves a change in amino acid 421 from valine to methionine (V421M) and the one in HTR2C involves a change in amino acid 23 from cysteine to serine (C23S). The rare polymorphic variants create unique restriction enzyme sites for TspR1 (V421M) in HTR2B and Hpy118I (C23S) in HTR2C for easy genotyping. To investigate both polymorphisms for possible modifier gene effects in TSC patients, we decided to genotype DNA from 490 phenotypically characterized TSC patients who had previously been tested by SSCP and/or direct sequencing for all exons of the TSC1 and TSC2 genes. Subtle mutations were identified in 77% (22% in TSC1 and 55% in TSC2) leaving 23% of the TSC-affected individuals with no mutation identified (NMI). After genotyping 237 individuals, it was discovered that only three individuals carried the V421M in HTR2B and we did not pursue this further. For HTR2C, 41 of 201 (20%) individuals tested to date have the 23Ser variant. Upon completion of genotyping, we will assess for genotype-phenotype correlation.

Molecular heterogeneity in segmental NF1. *L. Messiaen*^{1, 2}, *H. Brems*³, *O. Maertens*², *T. Callens*¹, *A. Helderman-van den Enden*⁴, *R. Sciot*⁵, *M. Flour*⁶, *B. Korff*¹, *E. Legius*³. 1) Dept Genetics, Univ Alabama Birmingham, AL; 2) Dept Medical Genetics, Univ Hosp Gent, Belgium; 3) Dept Human Genetics, Univ Hosp Leuven, Belgium; 4) LUMC, Leiden, The Netherlands; 5) Dept Pathology and; 6) Dept Dermatology, Univ Hosp St Raphael Leuven, Belgium.

We studied 3 patients who developed in adulthood multiple histology-proven cutaneous neurofibromas as the only NF1-related signs. We found 3 different mechanisms underlying their disorder. Somatic mosaicism for a NF1 total gene deletion was found in lymphocytes and neurofibroma-derived Schwann cells (SC) from the 1st patient. Selective culture of SC from 4 neurofibromas w/wo forskolin allowed identification of the 2nd hit in all tumors. In the 2nd patient, who presented with 2 discrete patches of cutaneous neurofibromas, a conserved missense mutation S139P was found in lymphocytes and fibroblasts. As this patient did not develop any NF1 related signs before his thirties, S139P may be a hypomorphic allele. In each of 6 neurofibromas studied, NF1^{+/+} SC carrying the S139P missense mutation only as well as NF1^{+/-} SC with a 4-bp deletion (6789-6792delTTAC) in addition to the S139P mutation were present. Fibroblasts from the neurofibromas only harbored the S139P mutation, indicating that 6789-6792delTTAC arose during development in a neural crest precursor cell. Furthermore, in all 6 neurofibromas studied, a different 2nd hit was found in the NF1^{-/-} SC cultures besides S139P and 6789-6792delTTAC. In the 3rd patient no NF1 mutation was found in the lymphocytes after comprehensive mutation analysis. However, a different 1st hit was found in the NF1^{+/-} SC cultures of each neurofibroma studied, indicating the presence of a mutator phenotype. A 2nd hit was identified in the NF1^{-/-} SC cultures of 2 out of 3 neurofibromas. None of these NF1 mutations was identified in blood lymphocytes. No microsatellite instability was observed in the neurofibromas. Further work is needed to understand the pathogenesis of this new condition. Although segmental NF1 is generally presumed to be a manifestation of somatic mosaicism for a NF1 mutation, the current study shows that the disorder is more complex and exhibits molecular heterogeneity.

Patients with Rothmund-Thomson syndrome and juvenile cataracts (Type I RTS) lack mutations in the *RECQL4* gene. L.L. Wang, R.A. Lewis, M.L. Levy, R. Davis, B. Chong, M. Hegde, S.E. Plon. Baylor College of Medicine, Houston, TX.

Rothmund-Thomson Syndrome (RTS) is an autosomal recessive disorder with a heterogeneous clinical profile characterized by skin rash (poikiloderma), small stature, sparse hair, gastrointestinal disturbance, skeletal abnormalities, and a significant risk for developing osteosarcoma. The original description of this disorder in 1868 by Auguste Rothmund, a German ophthalmologist, was notable for rapidly progressive bilateral juvenile cataracts in his patients. A recent study of a cohort of RTS subjects showed that two-thirds of subjects carried deleterious mutations in the *RECQL4* gene, which encodes a RECQ DNA helicase. Subjects carrying constitutional mutations in *RECQL4* were at a significant risk of developing osteosarcoma compared to subjects lacking *RECQL4* mutations. The previously studied cohort of RTS subjects had a low prevalence of cataracts (6 percent), and none developed the bilateral cataracts in childhood as described by Rothmund. We have subsequently identified four subjects with diagnostic features of RTS including bilateral cataracts diagnosed before the age of 6 years similar to Rothmunds patients. Three of the subjects were adults from the Mennonite community (two sisters ages 44 and 46 years and an unrelated 21 year old woman). The fourth subject was a 2 year old Caucasian child who also had small stature. All four subjects had the characteristic poikilodermatous rash, sparse or absent scalp hair, eyebrows and eyelashes, but none has developed cancer. DNA samples from the four subjects were evaluated by PCR and sequencing of the *RECQL4* gene, and no deleterious *RECQL4* mutations were identified. The Mennonite sibling pair was found to be discordant for multiple intragenic *RECQL4* single nucleotide polymorphisms, providing further evidence for genetic heterogeneity in this disorder. These data suggest that RTS subjects with bilateral juvenile cataracts have the Rothmund (Type I) form of RTS, and that they are likely to carry mutations in a gene other than *RECQL4*. Further studies using homozygosity mapping of the Mennonite patients are aimed at identifying the gene responsible for this Type I form of RTS associated with juvenile cataracts.

Genetic Basis of Familial Hypercholesterolemia Among Filipinos. *E.C. Cutiongco*¹, *E.R. Punzalan*², *R. Sy*², *R. Santos*², *E. Fadrigulan*², *S. Gosengfiao*², *P.M. George*³, *A. Laurie*³. 1) Institute of Human Genetics, National Institutes of Health, University of the Philippines Manila; 2) Department of Internal Medicine, University of the Philippines College of Medicine, Manila, Philippines; 3) Canterbury Health Laboratories, Christchurch, New Zealand.

Almost all heart disease results from susceptibility genes, defective single genes or genes interacting with various environmental factors. Discovery of these genes has led to a better understanding of the various types of heart disease prior to its onset, and more importantly, to development of strategies for its intervention. Familial hypercholesterolemia (FH) ranks as one of the most common genetic disorders affecting about 1 in 500 persons. It is transmitted as an autosomal dominant disorder involving the low-density lipoprotein (LDL) receptor gene. It is characterized phenotypically by extremely elevated levels of plasma LDL-cholesterol, tendon xanthomas, arcus cornealis and premature onset of atherosclerosis. To date, approximately 700 different mutations have been described in the LDL-receptor gene. These mutations lead to impaired function of the receptor by preventing proper cellular binding and uptake of LDL-cholesterol. Most mutations occur rarely and often only in single families although there are mutations described as prevalent in certain ethnic groups. Most heterogenous populations, however, carry a wide variety of mutations. Screening of exons 1-7 of the LDL receptor gene using DHPLC and subsequent DNA sequencing were done for the 60 Filipino subjects included in the study. We report the presence of mutations (D69N, H562Y, C308Y) previously documented in either Chinese or Malaysians. Novel mutations (intron 2 190+4A>T 5splice donor; exon 10b A480E; intron 8 1187-10G>A 3 splice acceptor) were also detected. This is the first study showing the heterogenetic basis of FH among Filipinos.

Normal and mutated fibrillin-1 alleles as modifiers of phenotypic severity in the Marfan syndrome. *M. Godfrey*^{1,2}, *J. De Backer*¹, *P. Van Acker*¹, *P. Coucke*¹, *A. De Paepe*¹. 1) Dept Medical Genetics, Ghent University Hospital, Ghent, Belgium; 2) Dept Pediatrics, Univ Nebraska Medical Center, Omaha, NE.

Background: The Marfan syndrome (MFS) is caused by mutations in the gene encoding fibrillin-1 gene (FBN1). Despite many hundreds of known FBN1 mutations no clear genotype/phenotype correlation has been identified. The high degree of inter- and intra-familial variability is likely due to a combination of genetic and environmental factors. It has been suggested that the expression of the normal FBN1 allele in affected individuals can influence the phenotypic severity of MFS. To test this hypothesis we studied the expression of the normal and mutant alleles in a large MFS family with high clinical variability.

Methods: Quantitative analysis of RNA expression in 10 affected members of the same family carrying a splice site mutation (IVS 8 -1 G>C) was performed on an ABI 3100 prism. The clinical severity in each individual was estimated using a grading system in which the phenotypic severity in each organ system was scored.

Results: Analysis showed the presence of 1 normal and 2 abnormal fragments. Sequencing of the abnormal fragments demonstrated that the splice site mutation creates both exon skipping (exon 9) and alternative splicing (in-frame deletion of 12 basepairs: c989-1000delATGTTCCGCCAG; p331-334del). The ratios of both normal and alternatively spliced fragments to the exon skipped fragment shows a possible relationship between their relative expression and clinical severity. The higher the expression of the normal and alternatively spliced fragments, the milder the phenotype. The higher the expression of the exon skipped fragment, the more severe the phenotype.

Conclusions: There appears to be a modifying effect on the severity of the phenotype by both the normal and mutant alleles. Patients with milder phenotypes appear to have higher expression levels of both the normal and alternatively spliced fragments compared to the exon skipped fragment. A more thorough examination for alternative spliced fragments should be considered in cases with mis-splicing mutations.

Study of the modifying role of androgens and androgen receptor in cardiac hypertrophy. *B. Gopinath, R.J. Trent, B. Yu.* Molecular and Clinical Genetic, Royal Prince Alfred Hospital, Sydney, NSW, Australia.

Cardiac hypertrophy is associated with increased cardiovascular morbidity and mortality, and it has been suggested that testosterone may be important in modulating cardiac mass. The steroid 5- reductase type 2 gene (*SRD5A2*) is a potential modifier, as it is the major enzyme that converts testosterone to dihydrotestosterone. The V89L change in *SRD5A2* has been found to be associated with lower levels of testosterone (Schatzl et al. 2002). Additionally, androgen receptors (AR) are present in myocardial tissue, which may permit androgens to modify the hypertrophic process via direct receptor-specific actions. In this study, we determined the distribution of the *SRD5A2* SNP (V89L) in 158 probands with familial hypertrophic cardiomyopathy (FHC) and 298 controls and characterised AR expression in a rat model with cardiac hypertrophy. This model was subsequently used to study the effects of testosterone stimulation on AR and one of its target genes, the β -myosin heavy chain gene. We found the V89L change in *SRD5A2* to be twice as frequent in FHC cases (10.8%) compared to controls (5.0%), but this was not significant perhaps due to small numbers tested. AR mRNA levels were 11-fold lower in neonates with cardiac hypertrophy compared with controls at postnatal day 1 (P 0.01). Testosterone treatment resulted in a slight increase in β -myosin heavy chain expression in both control and cardiac hypertrophic neonates, but this was not significant. In summary, the V89L change was higher in FHC cases although not conclusive. Further studies of *SRD5A2* haplotypes may confirm its role in cardiac hypertrophy. In our rat model, a unique decrease in AR expression was found in neonates with cardiac hypertrophy.

Lymphocytic Vasculitis in the Vaso Vasorum in Thoracic Aortic Aneurysms in patient with marfan syndrome, familial and sporadic disease: restricted usage of T-cell receptor BV genes suggests an autoimmune vasculitis.

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The histopathologic abnormality underlying ascending thoracic aortic aneurysm (TAA) was thought to be due to noninflammatory loss of smooth muscle cells and elastic fibers. The mouse model of Marfan syndrome (MFS) suggested that inflammation was a component of the aortic disease. We hypothesized that an autoimmune mechanism may be involved in the recruiting T lymphocytes and contributing to the pathogenesis of TAA. Aortic specimens were obtained from patients undergoing surgical repair of TAA with MFS (n = 9), FTAA (n = 7), sporadic TAA (n = 15), along from aortas from age matched controls (n = 6). Immunohistochemistry indicated that CD3+ cells significantly increased in the aortic media of TAA patients compared to those of controls (p<0.001) and in aortic media of patients with MFS and FTAA compared to those of sporadic TAA (p<0.05). In addition, CD68+ cells were also increased in the aortic media in the patients compared with controls. The number of infiltrating T lymphocytes and macrophages in the aortic media of TAA aortas were inversely correlated to the age of patients at the time of the repair (r = -0.83, p value <0.001; r = -0.70, p value <0.01, respectively). The gene expression profiles of T cell receptor chain variable region (TCRBV) was investigated using the real-time quantitative RT-PCR. The results indicated that there was a highly restricted use of BV22 and/or BV25 in the T cells in the aneurysm aortas, while control aortas and peripheral blood had diverse distribution of BV usage. The restricted usage of TCR BV gene by infiltrating T cells in the aortas with ascending aortic aneurysms suggest that autoimmune mechanism may be involved in the pathogenesis of TAA from patients with MFS, FTAA, and sporadic TAA.

Establishing the disease gene for TAAD1. *P. Gupta, D.G. Guo, D.M. Milewicz.* Dept Internal Medicine, Univ Texas Houston Med Sch, Houston, TX.

Ascending thoracic aortic aneurysms leading to aortic dissections (TAAD) can be inherited in an autosomal dominant inheritance in the absence of an associated syndrome. We have mapped a major locus for this disorder to 5q13-14, TAAD1, encompassing nearly 6cM. This is a major locus for TAAD based on studies using American and Finnish families, and this locus is responsible for up to 40% of all familial TAADs. The aim of this study is to identify the disease gene at the TAAD1 locus. Methodology used was direct sequencing in the forward and reverse direction of exons, as well as the splicing sites and intronic region adjacent to the exons. Using the public databases, we were able to identify 26 currently known genes according to RefSeq. Gene prediction programs like Twinscan, Genscan, MGC, etc identified a larger number of coding regions. These genes and coding regions were prioritized based on the known or putative function of the gene. The first genes to be sequenced were those involved in the extracellular matrix (ECM) because Marfan syndrome, a syndrome with TAAD, is due to mutations in fibrillin 1, an ECM protein. We sequenced versican, thrombospondin 4, SCAMP1, EDIL3, and cmya5, all of which are known ECM proteins or interact with the EDM, but known disease causing mutations were identified. Subsequently genes that are implicated in the intracellular protein trafficking were sequenced. These genes included HOMER1, TPO1, APG10L, and JMY. No disease causing alterations were identified in this set of genes. The next group of genes targeted was transcription factors, and LHFPL2, PAPD4, ZFYVE16, NYD-TSP1, and ZCCHC9 were sequenced. Finally, we investigated membrane bound proteins including RASGRF2, ZFYVE16, MGC23909, and UNQ9217, however, no disease causing mutations were identified. We are also identifying splice variants of all of these genes and sequencing the alternative exons. Other genes and coding regions have been selected based on known vascular expression patterns. We sequenced all of the exons in each known and predicted gene in the forward and reverse direction. While we have not identified the gene that is responsible for TAAD, we have found a number of novel SNPs not in the current databases.

No live individual homozygous for a novel endoglin mutation in a consanguinous Arab family with hereditary hemorrhagic telangiectasia. *A. Karabegovic*^{1, 2}, *M. Shinawi*³, *U. Cymerman*², *M. Letarte*^{1, 2}. 1) Immunology, University of Toronto, Toronto, Ontario, Canada; 2) Cancer Research Program, The Hospital for Sick Children, Toronto, Ontario, Canada; 3) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas.

Hereditary Hemorrhagic Telangiectasia (HHT) is an autosomal dominant vascular dysplasia characterized by dilated vessels and arteriovenous malformations (AVMs). It is associated with mutations in the Endoglin (ENG; HHT1) or Activin-like receptor kinase-1 (ALK1; HHT2) genes. Mice expressing a single allele of either of these genes can develop signs of disease whereas embryos lacking both alleles die at mid-gestation of cardiovascular defects. We have performed mutation analysis in a large Arab family with a known history of HHT and consanguinity. A novel ENG exon 7 missense mutation (c.932T>G) was found in the proband, who suffered from daily epistaxis and pulmonary and cerebral AVMs. The mutation was also present as a single allele in ten other individuals (age 5-56) with clinical signs of disease and an infant without symptoms. No mutation was found in 21 unaffected family members, strengthening the genotype/phenotype correlation. Marriage between two affected first cousins yielded five children, four affected and one normal. Two miscarriages occurred at 6-8 weeks of gestation. We propose that these fetuses were homozygous for the mutant allele, and died in utero at a time when endoglin is essential for cardiovascular development.

Screening for mutations in the gene encoding the regulatory protein FKBP12.6 in Finnish patients with catecholaminergic polymorphic ventricular tachycardia. *P.J. Laitinen^{1,2}, H. Swan³, K. Kontula^{1,2}*. 1) Research Program in Molecular Medicine, University Helsinki, Helsinki, Finland; 2) Department of Medicine, University of Helsinki, Helsinki, Finland; 3) Department of Cardiology, University of Helsinki, Helsinki, Finland.

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a severe inherited cardiac arrhythmic disorder. CPVT is highly penetrant and manifests with exercise-induced episodes of polymorphic ventricular tachycardias and sudden deaths. The onset of the symptoms is in adolescence or young adulthood and no structural abnormalities of cardiac muscle can be observed. If left untreated, CPVT results in high mortality. We previously mapped the gene underlying autosomal dominant CPVT to chromosome 1q42-43 and consequently identified mutations in the gene coding for cardiac calcium release channel, the ryanodine receptor (RYR2). Soon thereafter, the autosomal recessive form of the disease was shown to result from mutations of cardiac calsequestrin gene (CASQ2), which encodes an intrasarcoplasmic calcium storage protein. Even though mutations in RYR2 and CASQ2 account for a significant portion of familial CPVT, many families affected with CPVT do not harbor a mutation in either of these genes. FKBP12.6 (encoded by the FKBP1B gene) is a regulatory protein of RYR2 channel. We considered the FKBP1B gene a particularly intriguing candidate as defective RYR2:FKBP12.6 interaction has recently been suggested to occur in heart failure and CPVT. Therefore we aimed at investigating whether genetic alterations of the FKBP1B gene contribute to the molecular basis of CPVT in Finnish patients. Thirty index patients affected with CPVT were included in this study. We directly sequenced the complete coding region and exon flanking sequences of the FKBP1B gene. Several intronic SNPs, but no obvious disease-causing mutations were detected. We are currently determining the frequencies of these SNPs in a control cohort. In conclusion, the gene encoding the regulatory protein FKBP12.6 appears to play a minor, if any, role in the molecular pathogenesis of the Finnish CPVT patients included in this study.

Genetic Modifiers of Alagille Syndrome. *R. McDaniel¹, J.J.D. Morrissette¹, B.M. Kamath¹, M. Devoto², N.B. Spinner¹.* 1) Div. Human Genetics, Children's Hospital of Phila., Philadelphia, PA; 2) The Nemours Childrens Clinic, Wilmington, Delaware.

Alagille syndrome (AGS) is a complex dominant, multi-system disorder involving the liver, heart, and other systems. Penetrance of JAG1 mutations is close to 100%, but expressivity is highly variable both between and within families. Mutations in Jagged1 (JAG1), a ligand in the Notch signaling pathway are found in over 90% of patients. The Notch signaling pathway is critical for determination of cell fate decisions during development. Liver disease in AGS is a consequence of intrahepatic bile duct paucity, and ranges from minimal liver test abnormalities to end stage liver disease requiring transplantation. Possible contributors to variability include genetics factors (e.g. variation in the normal JAG1 allele or in other genes downstream of JAG1) and environmental factors. To study the contribution of allelic variation of JAG1 and other members of the Notch signaling pathway, we identified 13 families with multiple affected siblings. All had inherited a mutant JAG1 allele from an affected parent. We stratified family members according to disease severity and genotyped them to determine which JAG1 allele they inherited from their unaffected parent. To date, stratification and genotyping are complete for 7 JAG1 polymorphic markers in 7 families. Six of 7 sib pairs were discordant for both liver phenotype (one mild and one severe) and JAG1 genotype (i.e. they inherited different alleles from their unaffected parent). In the 7th sib pair, the male sibs had a maternally inherited JAG1 mutation, but they inherited different JAG1 alleles from their unaffected father. Both siblings had moderate to severe liver disease, although only one sibling required a transplant. These studies provide preliminary support for the hypothesis that polymorphisms on the non-disease JAG1 allele influence severity of liver disease, although the numbers are small and analysis is continuing. Similar analysis is being carried out for alleles of other potential modifier genes (e.g. Notch1 and Notch2 receptors).

Investigation on mutations of the MYH7 gene in Iranian hypertrophy cardiomyopathy (HCM) patients. *M. Montazeri¹, M. Ghani Kakhki^{1,3}, G. Estahbanati², DD. Farhud³, F. Nouhi², M. Peyghambari², N. Givtaj², M. Houshmand¹.* 1) Medical Genetic Department, National Institute for Genetic Engineering and Biotechnology, Tehran, Iran P.O.Box:14155-6343; 2) Tehran heart Hospital (Shaheed Rajaei); 3) Tehran University of medical Sciences, School of Public Health.

Hypertrophy cardiomyopathy (HCM) is a genetic disorder typically inherited in an autosomal dominant fashion with variable penetrance and variable expressivity. The disorder has a variable presentation and carries a high incidence of sudden death. The disorder has been estimated to occur in 0.05-0.2% of the population. While still in the early stage of research development, genetic testing can be used to identify a symptomatic family member with the same mutation as the proband (i.e. index case). HCM may occur at any age from newborn to elderly. In this study our patients have been selected according to their history (sudden cardiac death, physical signs, electrocardiography, cardiac catheterization and in some cases electro physiology. Our investigation is focused on mutation detection in the bMHC gene in exons that commonly have mutations. PCR is carried out using 13F and 15R primers and 19F and 21R primers to amplify 847bp and 799bp fragments, respectively. These amplimers were sent for sequencing. Other exons which have more common mutations such as exons 13, 14, 15, 19, 20 and 21 were sequenced. If there is a high-risk mutation in the patient, s family they would be referred to cardiologists for implanting ICD or drug therapy. In this study 52 HCM affected patients have been evaluated for mutations in exons 13-14-15-19-20-21 of the MYH7 gene with the aid of PCR and sequencing. Results showed that 14 of 52 patients had mutations after study their sequencing chromatogram. Four of these 14 patients had mutation in exonic regions and the other patients in the intronic region. Founded exonic mutations in patients were G10I95A in exon 13, A1041C in exon14, C13430T, C13978A in exon 19. Further study of patients families is needed to reveal the role of these mutations. It seems that mutation diagnosis in the MYH7 gene can give a good prognosis to prevent sudden death of affected ones.

Cardiac specific missense mutations in Jagged1: dosage or differential signaling? *J.J.D. Morrissette¹, D.A. Ross², E. Goldmuntz³, N.B. Spinner¹*. 1) Div Human Genetics, Children's Hosp Phila; 2) Dept Genetics, U. Penn School of Medicine; 3) Div. of Cardiology, Dept of Pediatrics, Children's Hosp Philadelphia, Phila, PA 19104.

Haploinsufficiency for the Notch Signaling Pathway ligand Jagged1 (JAG1) causes Alagille syndrome (AGS), a variably expressed, pleiotropic, dominant disorder. Liver and cardiac disease accounts for most of the morbidity in this disorder. JAG1 mutations have also been implicated in the etiology of isolated cardiac disease leading us to carry out a mutation screen of 126 patients with tetralogy of Fallot and 50 patients with pulmonic stenosis in which we identified 3 unique JAG1 missense mutations. We present molecular analysis of these mutations. The mutations (P810L, C664S, R937Q) were analyzed for their subcellular localization and ability to signal the Notch receptor. P810L did not reach the cell surface, and was inactive, similar to AGS associated missense mutations. However, C664S and R937Q retained partial activity, and some protein reached the cell surface. This was similar to previous findings on the mutation G274D, associated with dominantly inherited, isolated cardiac disease. To study downstream effects of decreased JAG-Notch signaling, quantitative RT-PCR experiments were carried out to assay levels of HES1 and HRT1, two genes activated by functional JAG1. There was differential activation of these genes, depending upon the mutant. Both HES1 and HRT1 were expressed in cells containing the Notch receptor, after stimulation with the C664S. However, the R937Q mutant differed in that HES1 was activated, but activation of HRT1 was greatly reduced. Since the HRT genes are considered essential regulators in heart development, loss of activation may be important in the development of heart disease. We suggest that the developing heart is more sensitive than other organ systems to decreases in JAG1 dosage. This may be due to different thresholds for transcriptional activation in heart sensitive tissues or the missense mutations in individuals with isolated cardiac disease may differentially signal to the various Notch receptors, leading to a cardiac specific phenotype.

Identification and characterization of a novel mutation in SCN5A and a common variant carrying risk for arrhythmia in Han Chinese population. *D. Niu*^{1,2}, *H. Hwang*³, *B. Hwang*¹, *S. Lau*¹, *C. Liao*¹, *J. Chen*³, *R. Shieh*³, *J. Wu*³, *Y. Chen*³. 1) Dept Pediatrics, Taipei Veteran General Hospital, Taipei; 2) Inst Clinical Medicine, National Yang-Ming University, Taipei, Taiwan; 3) Inst Biomedical Sciences, Academia Sinica, Taipei, Taiwan.

A four-generation family with five instances of sudden cardiac death was identified. One of the surviving family members received pacemaker insertion because of severe bradycardia. Other affected family members have cardiac conduction abnormalities such as prolonged PR interval and/or prolonged QRS duration. In all family members who underwent clinical evaluation, there were no signs of Brugada syndrome on resting ECGs, and structural abnormalities were excluded by conventional echocardiography. Using candidate gene approach, direct sequencing of SCN5As coding region (28 exons) revealed that two nucleotide changes present in this family. The first one, a G-to-A transition, leads to a non-synonymous amino acid change of R1192Q. The other, also a G-to-A transition, leads to a nonsense mutation (W1420X) and results in a shortened polypeptide. While the former was previously reported to be associated with Brugada syndrome and acquired long QT, the latter was a novel mutation and found to be associated with all affected members of this family. These two mutations were screened in ninety-four randomly selected Han-Chinese subjects. The nonsense mutation was not detected in any of the control subjects; however, the R1192Q mutation was present in 11% of the subjects with allele frequency of 0.06. Of those nine R1192Q carriers available for further cardiac studies, two had electrocardiographic abnormalities. One had frequent ventricular premature complexes and the other had prolonged QT interval. We concluded that W1420X mutation in the SCN5A is the cause of sudden death/cardiac conduction defects in this family. However, R1192Q, a Brugada/long QT mutation reported in other population, is a polymorphism in Han Chinese and individuals carrying the mutation may have an increased risk for arrhythmia.

Characterization of a mouse model for Hutchinson-Gilford Progeria Syndrome. R. Varga¹, M.R. Erdos¹, R. Virmani², F.D. Kolodgie², H. Avallone², W. Hagen³, E.G. Nabel⁴, F.S. Collins¹, M. Eriksson⁵. 1) NIH/NHGRI Genome Technology Branch, Bethesda, MD; 2) Armed Forces Institute of Pathology, Department of Cardiovascular Pathology, Washington, DC; 3) University of California at Irvine, Department of Medicine, Irvine, CA; 4) NIH/NHLBI Cardiovascular Branch, Bethesda, MD; 5) Karolinska Institutet, Department of Medical Nutrition, Huddinge, Sweden.

Hutchinson-Gilford Progeria Syndrome (HGPS) is a rare disorder characterized by symptoms of accelerated aging such as alopecia, growth retardation, sclerodermal skin changes and loss of subcutaneous fat. The average age of death is 12 years, commonly due to myocardial infarction or stroke. Cardiovascular pathology includes vascular smooth muscle cell depletion and myocardial hypertrophy and fibrosis. We recently discovered a *de novo* mutation, 1824C>T (G608G), in the lamin A gene (LMNA) in 90% of classical HGPS cases that creates a novel splice donor site in exon 11 (Eriksson et al., *Nature*, 423: 293-298, 2003).

In order to characterize this mutation's effects *in vivo*, mouse models have been created using two different strategies. In one approach, a 164 kb human BAC containing the LMNA gene has been recombineered in a bacterial host to incorporate the 1824C>T (G608G) mutation. In a second approach, a LMNA minigene is under the control of a tetracycline-regulated promoter that limits expression to specific tissues.

RT-PCR and western blotting from tissues of G608G BAC transgenic lines have revealed expression of the mutant LMNA RNA and protein gene products. Two founders died early without offspring and display pathologic changes in the heart and blood vessels that may indicate a progeric phenotype due to the mutant LMNA transgene. Histologic analysis revealed cardiac hypertrophy and fibrosis as well as arterial medial thickening in the heart and kidney.

These data provide interesting evidence of an analogous phenotype in these mice as compared with published data on human HGPS patients and suggest that these mice may provide a good model for studying accelerated atherosclerosis.

A locus for autosomal dominant isolated noncompaction of the ventricular myocardium maps to chromosome 11.

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Isolated noncompaction of the ventricular myocardium (INVM) is a cardiomyopathy characterized by loose, trabeculated myocardium, thought to arise from a block in the process in which myofibrils compact during cardiogenesis. Clinical manifestations are variable; some affected individuals are asymptomatic whereas others may develop ventricular dysfunction or arrhythmias. INVM is genetically heterogeneous; mutations in *G4.5/TAZ* on Xq28, *DB* on 18q12 and *ZASP/LDB3* on 10q22-q23 have been found in a small proportion of patients. Recently, genetic linkage to 11p15 has been reported in a single family. Thus, the etiology for most cases is still unknown. This report describes clinical and molecular studies of two unrelated families with INVM: the first consists of affected twin girls who carry a balanced translocation involving chromosomes 10 and 11, and the second, a four-generation family with multiple affected members. To identify the disease-causing gene, we mapped the chromosomal breakpoint in the first family and conducted genetic linkage analysis in the second family. By FISH using BACs and long range PCR-derived probes, the breakpoints on chromosome 10 and 11 have been localized to approximately 30 kb and 10 kb regions, respectively. Linkage analysis using markers spanning the chromosomal regions demonstrate strong linkage (LOD 3) with chromosome 11. The interval size varies from 15 cM to 50 cM depending upon how individuals with milder phenotypes are classified; all possible intervals include the breakpoint region. Our results provide evidence for a common genetic etiology in the two families and suggest that an additional locus for INVM exists on chromosome 11. Determination of the genetic basis will enhance our understanding of INVM and cardiogenesis.

Aceruloplasminemia Causing Brain Iron Overload and Dementia. *D.G. Brooks¹, J. Dunaief, MD, PhD², J.F. Schenck, MD, PhD³, E.A. Zimmerman, MD⁴.* 1) Div Medical Genetics, Univ Pennsylvania, Philadelphia, PA; 2) Dept Ophthalmology, Univ Pennsylvania, Phila, PA; 3) General Electric Research, Schenectady, NY; 4) Neurology, Albany Medical College, Albany NY.

Loss of function mutations in the ceruloplasmin (Cp) gene cause the rare, adult-onset disease aceruloplasminemia (aCp). Deficiency of Cp, the major serum and astrocyte ferroxidase generating Fe³⁺ for binding to apotransferrin, there is impaired iron recycling and iron-deficiency anemia. Excess iron accumulates in the pancreas, retina and brain contributing to the hallmark clinical triad of diabetes plus degeneration of brain and retina. A 57 year old man was evaluated for aCp after presenting with diabetes, macular degeneration, dementia and iron-deficiency anemia in spite of marked liver iron overload. Serum Cp was below the limit of detection in the proband and at half normal levels, consistent with recessive inheritance, in 3 healthy first degree relatives: father, sister and daughter. MRI showed markedly decreased signal in brain, liver > pancreas consistent with heavy iron accumulation. Mean T2 values were more than three standard deviations below the means of age-matched controls in caudate, putamen and hippocampus (p<0.0001) and markedly reduced in other regions including white matter and thalamus. Fundoscopic evidence of drusen and hyperpigmentation in both maculas were consistent with sub-clinical macular degeneration. Mutation analysis of the Cp gene is ongoing; the proband carries a C282Y mutation at the HFE locus which might protect against iron-deficiency anemia and/or contribute to his iron overload. This case emphasizes that a treatable risk factor for disease, iron overload, underlies three common conditions: dementia, diabetes and macular degeneration. Chelation and antioxidant therapies are ongoing however available iron chelators do not offer optimal penetration of the blood brain barrier. By presenting with dementia, this case highlights the need for better drugs to rid the brain of excess iron and/or neutralize the effects of redox-active metals in the brain. (Study Supported by: Neurosciences Institute of Albany Medical Center and GE Global Research).

Long QT syndrome: Identification and characterization of compound heterozygous patients. *H. Fodstad¹, M. Vukicevic³, J-B. Rougier³, I. Gautschi³, H. Abriel³, L. Schild³, K. Kontula¹, H. Swan².* 1) Biomedicum Helsinki, University of Helsinki, Finland; 2) Department of Cardiology, University of Helsinki, Finland; 3) Institute of Pharmacology and Toxicology, University of Lausanne, Switzerland.

Long QT syndrome (LQTS) is an inherited arrhythmic disorder, characterized by a prolonged QT interval on the ECG, ventricular arrhythmias and risk of sudden death. To date mutations in six voltage-gated cardiac ion channels, or the adaptor protein ankyrin B, have been shown to cause LQTS. Phenotypic variability exists both within and between families carrying the same mutation. Comprehensive knowledge of the underlying mechanisms for this variability is missing. The aim of this study was to search for possible compound heterozygous LQTS patients among affected Finnish families, and to find out whether this status contributes to phenotypic variation. We have identified three common mutations: A185T and R190G in the SCN5A gene, and R176W in the HERG gene. These mutations were studied in an extended cohort of 563 suspected LQTS families. In altogether 127 (23%) of these families we had previously detected an apparent disease-causing mutation in one of the LQTS genes. In seven families we identified twelve subjects who, in combination with an established LQTS mutation, had either the HERG R176W or the SCN5A R190G mutation. In addition, SCN5A A185T was found in eight, SCN5A R190G in three and HERG R176W in eight families as the sole mutation. The SCN5A A185T and R190G mutations alone seem to associate with normal QTc intervals (<440 ms), suggesting that their clinical impact on LQTS phenotype is limited, or that they represent some alternative and yet unknown disease mechanism. In contrast, the HERG R176W mutation alone seems to prolong the QTc interval: the QTc intervals in 37 mutation carriers versus 50 non-carriers were 44039 ms vs. 41022 ms, respectively. Functional in vitro studies of the SCN5A R190G and HERG R176W mutations are presently ongoing. Additional studies are required to clarify the impact of the compound heterozygosity on the LQTS phenotype and clinical outcome.

A novel heterozygous cDNA change in the Mre11 gene affects the stability of the MRN complex, ATM-dependent events, and karyotyping. *K.M. Gamo, S.A. Nahas, H.H. Chun, L. Eng, N.P. Rao, R.A. Gatti.* Department of Pathology, The David Geffen School of Medicine at UCLA, Los Angeles, CA.

Following exposure to ionizing radiation (IR), the ATM protein and the Mre11/Rad50/Nbs1 (MRN) complex play crucial roles in the cellular response to DNA double strand breaks (DSBs). In addition to the role of Mre11 as a substrate of ATM, it has recently been suggested that the MRN complex is involved in recruiting ATM to the site of DNA damage, where phosphorylation of ATM protein targets occurs. Here, we present an individual with a novel heterozygous cDNA change in the Mre11 gene (469delGTAA). While this individual does not display any clinical symptoms associated with either ataxia-telangiectasia (A-T) or ataxia-telangiectasia like disorder (A-TLD), we have observed an abnormal cellular phenotype including reduced expression of nuclear ATM and Mre11, reduced ATM kinase activity, *in vitro* radiosensitivity, impaired S-phase arrest, reduced Mre11 and phospho-Nbs1 (S343) IR-induced foci (IRIF), and chromosomal instability (t7;14). Thus, we demonstrate that even a heterozygous change in Mre11 can lead to definitive cellular aberrations, such as *in vitro* radiosensitivity and translocations of chromosomes 7 and 14.

Aprataxin gene mutations in a cohort of patients presenting with infantile onset cerebellar ataxia. *C. Gellera¹, S. Caimi¹, D. Di Bella¹, B. Castellotti¹, T. Granata², I. Moroni², G. Uziel², C. Mariotti¹, S. Di Donato¹, F. Taroni¹.* 1) Dept. of Biochemistry and Genetics, Istituto Nazionale Neurologico Besta, Milano, Italy; 2) Dept. of Child Neurology, Istituto Nazionale Neurologico Besta, Milano, Italy.

Ataxia with oculomotor apraxia type 1 (AOA1) is an autosomal recessive disease characterized by early-onset and slowly progressive cerebellar ataxia, areflexia, and peripheral neuropathy. Ocular apraxia is most prominent in early stage of the disease, by contrast, hypoalbuminemia, hypercholesterolemia and cognitive impairment are present in adult stage. AOA1 is caused by mutation in the APTX gene (9p13.3) encoding a nuclear protein (aprataxin) involved in the mechanism of DNA repair. We screened 130 unrelated patients (96% of Italian origin) for mutations in the APTX gene, including 110 sporadic cases and 20 patients presenting an autosomal recessive pattern of inheritance. All patients had progressive cerebellar ataxia with onset before 25 yrs., and were negative for the Friedreich ataxia GAA expansion. Mutational screening was performed by DHPLC analysis of exons 5, 6 and 7, coding for two functional motif of the protein, where the APTX mutations have been identified so far. We identified APTX gene mutations in aprox. 6% of cases: 10 patients belonging to 8 unrelated families (7 from Italy and 1 from Pakistan). Six patients belonging to 4 families carried the most frequent nonsense mutation identified so far: the W279X in homozygous form; 2 unrelated patients carried two different previously described missense mutation in homozygous form: the W279R and P206L; while 2 other unrelated patients carried two newly identified nonsense mutations: the R247X in homozygous form in a patient from Pakistan and the R306X in heterozygous form in a compound heterozygote patients carrying the W279X on the other allele. We report the identification of two novel nonsense mutations, one is the first mutation identified in exon 7. Our study indicate that APTX mutations are a relative frequent cause of disease in sporadic and recessive early-onset progressive ataxia. (Partially supported by grant Ministero Salute RF2002/160 to FT).

Mechanism and timing of mitotic rearrangements in the subtelomeric D4Z4 repeat involved in FSHD. *R.J.L.F. Lemmers¹, P.G.M. van Overveld¹, L.A. Sandkuij¹, H. Vrieling², G.W. Padberg³, R.R. Frants¹, S.M. van der Maarel¹.* 1) Human & Clinical Genetics, LUMC Leiden, Leiden, Netherlands; 2) 2.Department of Toxicogenetics, LUMC, Leiden, The Netherlands; 3) Department of Neurology, UMC, Nijmegen, The Netherlands.

Facioscapulohumeral muscular dystrophy (FSHD1A, MIM 158900) is an autosomal dominant myopathy displaying large inter- and intrafamilial variability. FSHD is associated with contractions of the polymorphic D4Z4 repeat on chromosome 4qter. Almost half of the new FSHD mutations occur post-fertilization, resulting in somatic mosaicism for D4Z4. Detailed D4Z4 analysis of eleven mosaic FSHD individuals revealed a mosaic mixture of a contracted FSHD sized allele and the unchanged ancestral allele in eight cases, which is suggestive for a mitotic gene conversion without crossover. However, in three cases the D4Z4 rearrangement resulted in two different sized D4Z4 repeats, indicative for a gene conversion with crossover. In all cases DNA markers proximal and distal to D4Z4 showed no allelic exchanges, suggesting that all rearrangements were intrachromosomal. We propose that D4Z4 rearrangements occur via a synthesis-dependent strand annealing (SDSA) model that relatively frequently allows for crossovers, which was recently confirmed by the detection of two other complex mosaic cases among eight new identified mosaic FSHD cases. Furthermore, the distribution of different cell populations in mosaic FSHD patients suggests that mosaicism here results from D4Z4 rearrangements occurring during the first few zygotic cell divisions after fertilization. Current investigations aim at the identification of chromatin factors associated with D4Z4 repeat instability.

Molecular analysis of the *LITAF/SIMPLE* and *PRX* genes in patients with demyelinating Charcot-Marie-Tooth (CMT) disease. *M. Milani*¹, *M. Cesani*¹, *S. Baratta*¹, *C. Caccia*¹, *M.R. Balestrini*², *D. Riva*², *J. Sepcic*³, *D. Pareyson*¹, *F. Taroni*¹. 1) Div of Biochemistry & Genetics, Ist Naz Neurologico C. Besta, Milan, Italy; 2) Div of Child Neurology, Ist Naz Neurologico C. Besta, Milan, Italy; 3) Dept of Neurology, University of Rijeka, Rijeka, Croatia.

At least 20 genetic loci and 13 genes have been associated with the demyelinating form of CMT disease (CMT1) and related disorders (DSD, CH). Mutations in the *LITAF/SIMPLE* gene (CMT1C; 16p13.1-12.3) have been recently suggested to be a relatively common cause of CMT1 after the CMT1A duplication and *GJB1/Cx32* mutations (Saifi *et al.*, ASHG 2003). We have screened for *LITAF* mutations by DHPLC and sequence analysis a group of 200 CMT1 patients negative for the CMT1A duplication and *MPZ*, *PMP22*, and *GJB1/Cx32* mutations. We have identified 2 relatively frequent polymorphisms, 3 rare polymorphisms, and 3 novel mutations. These latter were identified in heterozygous form in 3 unrelated adult patients (1 familial dominant case and 2 apparently sporadic cases) affected with moderate-to-severe forms of CMT1. Our data indicate that mutations in the *LITAF/SIMPLE* gene are not a frequent cause of demyelinating CMT.

Unlike the dominant forms, only a limited number of loci have been linked to the rare recessive forms (AR-CMT). The 4.9-kb coding region of the periaxin gene (*PRX*, CMT4F) was scanned for mutations by DHPLC and sequence analysis in a group of 30 CMT1A/*MPZ*/*PMP22*/*GJB1*-negative patients with severe demyelinating neuropathy. Three novel frameshift and nonsense mutations were identified in two AR families with 2 affected siblings in each family. Interestingly, in one family, the two siblings exhibited different disease severity and were found to be compound heterozygotes for two mutations affecting the long (L-periaxin) but not the short (S-periaxin) variant of the protein, suggesting that S-periaxin expression may account for clinical variability in these patients. [Supported by grants from Ministero della Salute (RF 2002/160) and Fondazione Mariani to F.T.].

Walker Warburg syndrome phenotype in Egyptian proband with mutation in the Fukutin gene. A. Pai¹, H. Van Bokhoven², G. Ryan¹, D. Chitayat¹. 1) Mount Sinai Hospital, Prenatal Diagnosis and Medical Genetics, Toronto, Canada Toronto, ON,; 2) Department of Human Genetics University Medical Center, Nijmegen, The Netherlands.

Walker Warburg syndrome (WWS) is an autosomal recessive disorder characterized by congenital muscular dystrophy, ocular and complex brain abnormalities. WWS along with muscle-eye-brain disease (MEB) and Fukuyama-type congenital muscular dystrophy (FCMD) are believed to share a similar pathomechanism. Clinical resemblance between the WWS, MEB and FCMD phenotypes can make diagnosis difficult. However, FCMD and MEB show striking Founder effects in Japanese and Finnish populations respectively, while WWS has been seen in different populations. At the molecular level, different MEB causing mutations have been identified in Japanese, Korean as well as Caucasian patients who were given diagnoses of either MEB or WWS or FCMD. We describe an Egyptian couple who had previously lost a child diagnosed with WWS. The couple presented to us prenatally with a recurrence WWS. Subsequent investigations performed in the neonatal period confirmed a clinical diagnosis of WWS with classic features of hydrocephalus, microphthalmia, hypotonia and cloudy cornea. DNA analysis revealed a homozygous frameshift mutation in the Fukutin gene, which has been associated with FCMD patients from the Japanese population. Genotype-phenotype correlations in this case are of particular interest due to the location of the mutation. The above case emphasizes the complexity of this group of congenital muscular dystrophies and makes a case for examining additional WWS patients who may also have mutations in the Fukutin gene versus the POMT1 gene in which mutations in WWS patients have already been described along with detailed genotype-phenotype correlations.

Allelic heterogeneity of NF1 gene mutations and phenotypic variations in 9 Korean NF1 kindreds. *S.J Park, H.J. Kim.* Medical Genetics, Ajou Univ. School of Medicine, Suwon, kunggi-do, Korea.

Neurofibromatosis type 1 (NF1) is one of the most common inherited disorders and is characterized by highly variable expressivity. The NF-1 gene is located at 17q11.2, contains 60 exons spanning approximately 350kb of genomic DNA, and encodes a 12kb transcript. The gene product, neurofibromin, has an estimated molecular weight of 327kDa and is widely expressed in many tissue. The mutation rate in NF1 gene is one of the highest reported human disorders. As of June 2004, the Human Gene Mutation Database(HGMD, Cardiff) listed more than 547 different NF1 mutations. The majority of them lead to a truncated protein product. To understand the molecular basis of phenotypic characteristics of Korean NF1 pts., we studied delineation of clinical phenotype and pedigree analysis of 14 pts. in 9 Korean family and NF1 mutation screening of 9 unrelated pts. by means of PCR, RT-PCR, microsatellite analysis and direct cycle sequencing for mutations in the whole coding sequence and the splice sites of the NF1 gene. 7 different mutations were identified in 3 sporadic and 4 familial cases with 6 single base substitutions (one missense, four nonsense and one splicing region mutation) and 1 single base insertion including 5 previous reported mutations (2446C>T R816X in exon 16, 4537C>T R1513X in exon 27, 3827G>A R1276Q in exon22, IVS8+1G>A and 6792C>G Y2264X in exon 37) and 2 novel ones (IVS21+2-3insT in exon 21 and 532G>T E178X in exon 4). Three mutations were detected in the GAP related domain(GRD) which is known functional domain of the NF1 gene and spans exons 20-27 and four mutations in exon 4, 8, 16 and 37. 9 affected members in 4 familial cases showed wide range of phenotypic variation. We found that affected members of same family sharing an identical mutant NF1 allele have different disease phenotypes. Intrafamilial phenotypic variation suggest that unlinked modifying genes may be involved in the development of particular clinical features of NF1.

Detection of Six Novel *RASA1* Mutations in Patients with Capillary Malformation-Arteriovenous Malformation.
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Capillary Malformation-Arteriovenous Malformation (CM-AVM) (MIM 608354) is a recently identified phenotype characterized by atypical capillary malformations (CM) associated with either arteriovenous malformation, arteriovenous fistula, or Parkes Weber syndrome (Eerola *et al.*, 2003). In this phenotype, CM are usually multiple, small, round to oval in shape and pinkish red in color. Eerola *et al.*, have shown that the CM-AVM phenotype is caused by mutations in the *RASA1* gene and reported 6 different mutations. The aim of this study was to consolidate the involvement of the *RASA1* gene in CM-AVM patients. For this, we have screened novel families exhibiting CM-AVM phenotype for mutations in the 25 exons of *RASA1* by single-strand conformational polymorphism and heteroduplex analyses. The fragments with abnormal migration were analyzed by sequencing. We identified 6 novel mutations in the *RASA1* gene: one deletion, one insertion of 3 nucleotides, 2 missense and 2 nonsense mutations. The deletion caused reading-frame shift and is predicted to result in a truncated protein. This data confirms that inactivating mutations in the *RASA1* gene are responsible for the CM-AVM phenotype. (vikkula@bchm.ucl.ac.be) (<http://www.icp.ucl.ac.be/vikkula>).

Study of the Ferritin light Polypeptide (*FTL*) gene, including the cis-acting Iron-Responsive Element (IRE), in Portuguese patients with Huntington-like symptoms. A. Teixeira-Castro¹, M.C. Costa¹, M. Constante-Pereira², J. Sequeiros³, M.M. Santos², P. Maciel¹. 1) ICVS/Health Sciences School, University of Minho, Braga; 2) Dept. Medicin, CHUM, Univ. Montreal, Canada; 3) UnIGENE, IBMC, Univ. Porto, Portugal.

Iron metabolism dysregulation has been associated with several neurological disorders. An insertion (460-461 InsA) in the coding region of the ferritin light polypeptide (*FTL*) gene was reported in patients of English and French ancestry causing neuroferritinopathy. These patients presented extrapyramidal symptoms, choreoathetosis, dystonia, spasticity and rigidity similar to Huntington disease (HD) but without cognitive decline, as well as low ferritin plasma levels. The content of cytoplasmatic ferritin is regulated by the translation of ferritin H and L mRNAs in response to an intracellular pool of iron by interaction between RNA binding proteins to the iron responsive element (IRE) localized on the conserved 5' UTR of ferritin H and L mRNAs, that appears as a stem-loop secondary structure. Several mutations have been described on the IRE of *FTL* (18 different nucleotide substitutions, two small deletions, two gross deletions and a double point mutation), all of these causing Hereditary Hyperferritinemia-Cataract Syndrome (HHCS). In order to verify (i) if mutations in the *FTL* gene explained the phenotype in a significant proportion of patients with movement disorders and (ii) whether mutations in the IRE of this gene could also be associated to neurological dysfunction, we assessed a group of 86 Portuguese patients with clinical manifestations partially overlapping with HD, in whom the CAG expansion in the HD gene had been excluded - HD-like patients. In our series, 50% of the patients had typical HD symptoms, whereas the remaining presented either only a movement disorder (77%) or only psychiatric alterations (23%). We have found one novel missense mutation in the *FTL* gene (474GA, leading to the A95T substitution) in a patient with early onset bilateral pallidal involvement and in his mother. No mutations have been found in the *FTL* IRE of the 86 patients, excluding this as a major cause of HD-like phenotype.

Identification of the Genetic Defect Causing Bleeding Disorder, East Texas Type (OMIM:605913). *L.M. Vincent, D.M. Milewicz.* Division of Medical Genetics, Dept of Internal Medicine, University of Texas-Houston Medical School, Houston, TX.

A novel bleeding disorder exhibiting an autosomal dominant mode of inheritance has been identified in a large kindred from east Texas (Bleeding Disorder, East Texas Type, OMIM:605913). The disorder is clinically characterized by easy bruising, life-threatening bleeding with trauma or surgery, and menorrhagia in affected women. Laboratory studies demonstrated prolongation of the prothrombin time (PT) and/or activated partial thromboplastin time (aPTT) in affected individuals. Paradoxically, assays of all known coagulation factors were within normal levels. Using candidate gene linkage, we mapped the critical interval containing the defective gene to a 13.4 Mb region on 1q24-25. After recruiting more individuals, we have further prioritized the critical interval to 7.8 Mb. We are systematically identifying and screening candidate genes for the disease causing mutation. To date, eleven candidate genes, including antithrombin III and coagulation factor V, have been sequenced, and no disease causing alterations have been identified. In addition, a biochemical assay has been developed in order to monitor the defect protein in plasma samples taken from affected individuals. This assay is being used to test the stability and activity of the defect after subjecting the affected plasma to varying experimental conditions. For example, after subjecting affected patient plasma to aluminum hydroxide, we found that the assay yielded normal results. Thus, we discovered that the defect binds either directly to aluminum hydroxide or indirectly to a protein that is vitamin K-dependent. When all proteins that bind to aluminum hydroxide were electrophoresed under reducing conditions, a novel 250kDa band was observed in all affected patients, but absent from controls. This band was excised, and is currently being sequenced. Elucidation of the genetic defect causing Bleeding Disorder, East Texas Type will advance our understanding of coagulation, potentially revealing a novel protein involved in the coagulation cascade, and may provide insights for novel therapy to delay or prevent coagulation.

Mutation analysis of *DNAI1* in patients with Primary Ciliary Dyskinesia (PCD). M. Zariwala¹, M.P. Kennedy¹, M.W. Leigh¹, P.G. Noone¹, R.U. de Jongh², L.M. Morgan², J. Rutland², J. Horvath³, H. Omran³, U. Prada⁴, R. Chodhari⁵, E.M.K. Chung⁵, H.M. Mitchison⁵, M.R. Knowles¹. 1) UNC-Chapel Hill, NC; 2) Concord Hospital, Sydney; 3) the Universitäts-Kinderklinik, Freiburg; 4) University of Verona, Verona; 5) Royal Free and University College Medical School, London.

Primary ciliary dyskinesia (PCD) is characterized by oto-sino-pulmonary disease and half the patients have situs inversus. It is usually inherited as an autosomal recessive trait. It is caused by abnormalities in the structure and function of cilia of respiratory tract and flagella of sperm. Ultrastructure analysis shows defective outer and/or inner dynein arms in ~ 90% of the patients. The disease is genetically heterogeneous and mutations have been reported in *DNAH5* (n=8 families) [Olbrich et al., Nat. Genet. 30: 143, 2002] and *DNAI1* (n=6 families) [Pennarun et al., AJHG; 65: 1508, 1999; Guichard et al., AJHG; 68: 1030, 2001; Zariwala et al., AJRCMB; 25: 577, 2001]. Both of these genes encode outer dynein arm (ODA) proteins. Genetic heterogeneity makes it challenging to identify disease-causing mutations in PCD patients. We analysed 78 unrelated PCD patients with ODA defects for genetic mutations in *DNAI1*. Thirteen families were excluded from linkage to *DNAI1* by using 'exclusion mapping'. The remaining 65 families were sequenced for all 20 exons. We identified 10 unrelated patients who harbored mutations in *DNAI1*. Six patients had mutations on both alleles, whereas 4 patients had mutations on one allele. Testing for splice mutation by RT-PCR is underway in these 4 patients. Of these 16 mutated alleles, 5 were novel mutations. Thus far, 121 patients (published and current study) have been tested, and 17 (14%) unrelated patients are known to harbor mutations. Despite locus heterogeneity, 15 mutant alleles carried the 219+3insT mutation and 6 mutant alleles were in exon 17. Since the majority of *DNAI1* mutations detected thus far are 219+3insT and in exon 17, screening PCD patients for these mutations can be used to expedite the mutation detection process and will help in devising a clinical test panel. This abstract is funded by GCRC #00046, M01 RR00046-42, NIH HL04225, NIH/NHLBI HL071798-01A1 and Wellcome Trust (UK).

Clinical and molecular characterization of Sotos syndrome in Spanish patients. *J. Del Valle*¹, *M. Del Campo*¹, *I. Delicado*², *E. Gabau*⁵, *F. Ramos*⁴, *C. Mellado*³, *I. Lopez Pajares*², *M.C. Roche*², *R. Gracia*², *L.A. Perez Jurado*¹, *P. Lapunzina*². 1) Genetics Unit. Dept of Experimental Science, Universitat Pompeu Fabra, Barcelona, Spain; 2) Genetics, Pediatric Neurology and Endocrinology, University Hospital La Paz, Madrid, Spain; 3) Dept of Pediatrics. Universidad Catolica de Chile, Santiago, Chile; 4) Dept of Pediatrics. Universidad de Zaragoza, Spain; 5) Dept of Pediatrics, Hospital Parc Tauli, Sabadell, Spain.

Sotos syndrome is an overgrowth syndrome characterized by pre and postnatal overgrowth, advanced bone age, developmental delay and mental retardation of variable severity, macrocephaly and a characteristic facial gestalt with a prominent forehead, apparent hypertelorism, downslanting palpebral fissures, receding hair line and pointed chin. The major cause of Sotos syndrome, an autosomal dominant condition, is haploinsufficiency for the NSD1 gene (nuclear receptor binding SET-domain 1). Microdeletions encompassing the NSD1 gene, likely mediated by unequal homologous recombination between flanking segmental duplications, are common in Japanese patients with Sotos syndrome, whereas point mutations in NSD1 have been predominant in all European published series. In this study, we report on a series of 18 Spanish and 2 Chilean patients with a firm clinical diagnosis of Sotos syndrome and molecular confirmation of NSD1 mutation. In search of common deletions, we used FISH and microsatellite markers inside and flanking the gene. Only two patients showed deletions encompassing NSD1. Screening for point mutations was performed using dHPLC and SSCP, and/or direct sequencing. Point mutations or small rearrangements have been identified in 18 additional patients. Sixteen are located in exons (>50% in exon 5), all but one causing either early stop-codons or frameshift, and 2 affect splicing signals, all predicting truncated proteins. None of these mutations have been previously reported, confirming the absence of mutational hotspots in European studies. In conclusion, this study confirms the clinical specificity to predict mutations in NSD1, the infrequent occurrence of deletions in European Sotos patients, and the mutational heterogeneity of the disorder.

Partial and variable recapitulation of the human BBS phenotype in the *Bbs4* null mouse. *E.R. Eichers*¹, *R.A. Lewis*^{1, 2, 3, 4}, *N. Katsanis*⁶, *J.R. Lupski*^{1,3,5}. 1) Depts of Mol & Human Genetics; 2) Ophthalmology; 3) Pediatrics and; 4) Medicine, Baylor Col of Med, and; 5) Texas Children's Hospital, Houston, TX; 6) Inst of Genetic Medicine and Wilmer Eye Inst, Johns Hopkins Univ, Baltimore, MD.

Bardet-Biedl syndrome (BBS) is a rare oligogenic disorder exhibiting clinical heterogeneity and complex inheritance. Although the BBS phenotype is variable both between and within families, the syndrome is characterized by mental retardation, polydactyly, obesity, hypogenitalism, renal abnormalities, retinal dystrophy, and several secondary features. Eight genes mutated in BBS patients have been identified. To investigate the etiopathogenesis of BBS, we created a mouse null for one of the *Bbs* genes, *Bbs4*, to analyze the contribution of one gene to the pleiotropic BBS phenotype in mouse.

Evaluations of these null mice have shown phenotypes with incomplete penetrance and various levels of expressivity, with partial recapitulation of the human phenotype. Heterozygous N2 matings have yielded 359 pups, with significantly distorted ratios (0.27 wild-type : 0.57 heterozygous : 0.16 null), suggesting partial embryonic lethality. Two of the 3 homozygous males and 3 of 3 females tested are fertile. Unlike the obesity characteristic of affected humans, all *Bbs4* null mice are significantly runted compared to their littermates when the animals are young. Most null mice attain a weight similar to their littermates over time, but several remain underweight. Only 3 null animals are obese (N = 38 (N2F1 and N2F2), age \geq 7 weeks). No null mice have polydactyly. We also observed ocular abnormalities in 7 of our homozygous mice, but these changes are variable in age of onset. No renal disease has been noted by autopsy.

Our data indicate that absence of *Bbs4* alone is insufficient to recapitulate fully the BBS phenotype, suggesting that additional alleles are required for the expression of specific phenotypic features. This interpretation is consistent with the variability seen within and between BBS patients and families and further supports the complex inheritance patterns in this syndrome and the possible influence of genetic background.

Analysis of atypical polydactyly patients: Surprisingly high yield of *GLI3* mutations in patients with mild Pallister-Hall and Greig cephalopolydactyly syndrome features. *J.J. Johnston, L.G. Biesecker, Clinical Collaborators. GDRB, NHGRI, Bethesda, MD.*

Mutations in the *GLI3* zinc finger transcription factor on chromosome 7p14.1 cause the Pallister-Hall (PHS) and Greig cephalopolysyndactyly (GCPS) syndromes. Mutations in *GLI3* have also been described in three types of apparently isolated polydactyly, PAP-A, PAP-A/B, and PPD-IV. It is unclear whether the apparently isolated polydactyly disorders represent the mild end of the disease spectrum for PHS and GCPS or if they are distinct clinical entities. In order to understand the clinical variability resulting from mutations in *GLI3* we have studied a cohort of patients who have features of PHS and GCPS. These patients do not fulfill the clinical criteria for either disorder. The group consisted of six individuals without preaxial polydactyly who had postaxial polydactyly and craniofacial features consistent with GCPS, and 12 individuals with postaxial polydactyly and features consistent with PHS including hypothalamic hamartomas and bifid epiglotti. In addition we analyzed four individuals with hypothalamic hamartomas without polydactyly (two isolated hamartomas and two with oral frenulae), and three individuals thought to have non-syndromic polydactyly. We identified a total of nine mutations and one missense alteration. Three individuals with postaxial polydactyly and craniofacial features consistent with GCPS had mutations in *GLI3* (50%) and another had a missense alteration that segregated with the disease in the family (17%). Six individuals with postaxial polydactyly and features consistent with PHS had mutations in *GLI3* (50%). Although the mutation yield is lower than that found in individuals who fulfill the diagnostic criteria (75%) mutation analysis in this set of patients suggests that clinicians should have a high index of suspicion for *GLI3* mutations in such patients. None of our patients with hamartomas in the absence of polydactyly, or isolated polydactyly, had mutations in *GLI3*. Our results suggest that individuals with polydactyly in combination with other features of PHS or GCPS should be screened for mutations in *GLI3*.

PTPN11 mutations in patients with LEOPARD syndrome : French Multicentric Experience. *B. Keren¹, Y. Sznajder¹, S. Saba¹, A. Hadchouel¹, D. Bonneau², B. LeHeup³, D. Lacombe⁴, A. Toutain⁵, S. Marlin⁶, G. Mortier⁷, C. Baumann¹, H. Cave¹, A. Verloes¹.* 1) Genetic Depts, Hopital R Debré, Paris; 2) Angers University Hospital; 3) Nancy University Hospital; 4) Bordeaux University Hospital; 5) Tours University Hospital; 6) Armand Trousseau Hospital, Paris, France; 7) Ghent University Hospital, Belgium.

LEOPARD syndrome (LS) is an autosomal dominant disorder characterized by Lentigines and caf-au-lait spots, EKG anomalies, Ocular hypertelorism, Pulmonary stenosis, Abnormal genitalia, Retardation of growth and Deafness. Insofar, 3 distinct mutations of the PTPN11 gene have been reported in 15 patients with LS. Screening of 14 unrelated propositi with a clinical diagnosis of LS from the French Multicentric Noonan Study yielded four different nucleotidic changes in PTPN11. The two previously described recurrent mutations Y279C in exon 7, and T468M in exon 12 were identified respectively in 6 and 4 unrelated patients. Two new mutations were identified. A substitution Y279S was found in 1 child who developed acute myeloid leukemia. A missense variation Q510P was found in 2 unrelated patients. Among 8 parents whose parental DNA was available, 3 mothers and 1 father were carriers and clinically affected. No mutations were found in 1 atypical, familial case who further exhibited marfanoid habitus. Comparing T468M and Y279C phenotype showed a significant difference for EKG anomalies, and a trend for heart defect, growth retardation, and deafness. AML, a probably not so rare complication of Noonan syndrome, is reported here for the first time with LS.

No evidence of SARA2 gene mutation in our patients with Marinesco-Sjogrens syndrome (MSS). *J. Lee¹, B. Chodirker², A.E. Chudley², S.R. Sanders², C. Carriles², C. Powell², J.J. Mulvihill¹, S. Li¹.* 1) Dept Pediatrics, BSEB #224, OUHSC Genetics Lab, Oklahoma City, OK; 2) Section of Genetics and Metabolism, Department of Pediatrics and ChildHealth, Children's Hospital, University of Manitoba, Winnipeg, Manitoba, Canada.

MSS is an autosomal recessive disorder with full penetrance that causes cerebellar ataxia, cataract, short stature, mental subnormality, hypergonadotropic hypogonadism and myopathy. Recently, SARA2 has been considered as a candidate gene responsible for MSS. However, no mutation was found in another study of 4 families with multiple affected individuals with a classical diagnosis of MSS. Because of contradictory data, we investigated 2 unrelated patients with MSS. Total genomic DNA was isolated and direct DNA sequencing utilizing primers covering all 7 exons was performed. No disease-causative mutation was found in either patient. A silent mutation was identified in one of the patients. The findings suggest that the SARA2 gene mutation is not the cause of MSS in our two patients.

Evidence for a second molecular mechanism resulting in the predominantly ocular form of Stickler syndrome : Alternative missplicing of a COL2A1 pathogenic mutation. *A.J. Richards¹, S. Meredith², P.W.P. Bearcroft², A. Poulson², G. Crossland², D.M. Baguley², J.D. Scott², M.P. Snead².* 1) Dept Pathology, University of Cambridge, Cambridge, United Kingdom; 2) Addenbrooke's NHS Trust, Hills Road, Cambridge, United Kingdom.

Type 1 Stickler syndrome is caused by mutations in COL2A1 the gene for type II collagen. It usually exhibits both eye and systemic features including myopia, retinal detachment, osteoarthritis, cleft palate and deafness. Certain mutations in exon 2 of COL2A1 result in a predominantly ocular form of the disorder, as this exon is alternatively spliced and excluded from the mature transcripts in cartilage. **Purpose:** To characterise the mutation in a family with predominantly ocular Stickler syndrome, but with a normal exon 2 sequence. **Methods and Results:** A family with the type 1 membranous phenotype of Stickler syndrome were assessed for skeletal and hearing traits associated with the disorder. None were found. Exon 2 of COL2A1 had a normal sequence. Mutation screening detected a T>C mutation converting the donor splice site of intron 51 from GT to GC. Amplification of illegitimate transcripts from cultured skin fibroblasts showed retention of intron 51 and premature termination of translation. Donor splice sites with GC dinucleotide sequences are often involved in alternative splicing. To determine if cartilage cells could splice this mutant allele normally, an expression vector containing minigenes with various mutant IVS51 donor splice sites were transfected into cultured cells. Mini genes with IVS51G+1T, G+1A and T+2C mutations were all misspliced (exon skipped) when transfected into cell lines derived from ocular tissue (MIO-M1, corneal, and scleral cells). Whereas the G+1T and G+1A mutants were misspliced in all cell lines derived from skeletal tissue, in contrast the T+2C mutant was spliced correctly in 2 of 3 (Saos-2 and SW1353) of these cell lines. **Conclusion:** The systemic features associated with Stickler syndrome are lacking in the family with the intron 51 T+2C mutation probably because cartilage cells are, at least partially, capable of splicing the mutant allele normally, whereas ocular tissue cannot.

Phenotype of SGCE mutations in a large sample of French patients. *S. Tezenas du Montcel*¹, *F. Clot*², *M. Vidailhet*^{2,3}, *P. Jedynek*⁴, *P. Damier*⁵, *F. Durif*⁶, *G. Ponsot*⁷, *C. Tranchant*⁸, *L. Vercueil*⁹, *A. Brice*². 1) Service de Biostatistique, Hopital Pitie-Salpetriere, AP-HP, Paris; 2) INSERM U289, Paris; 3) Service de Neurologie, Hopital Saint-Antoine, AP-HP, Paris; 4) Departement de Neurologie, Hopital Pitie-Salpetriere, AP-HP, Paris; 5) Service de Neurologie, Hopital Laennec, Nantes; 6) Service de Neurologie, Hopital Gabriel Montpied, Clermont-Ferrand; 7) Service de Neuropediatrie, Hopital Saint Vincent de Paul, Paris; 8) Hopital Universitaire de Strasbourg, Strasbourg; 9) Service de Neurologie, CHU de Grenoble, France.

Myoclonus Dystonia Syndrome (MDS) is an autosomal-dominant movement disorder characterized by rapid muscle contractions (myoclonus) and sustained twisting and repetitive postures (dystonia). The MDS gene (SGCE) encodes - sarcoglycan. We have evaluated the frequency of SGCE mutations and the corresponding phenotype in a large cohort of French patients with myoclonic syndromes. We performed SGCE screening in 74 index cases, half with family history of myoclonus or dystonia, with myoclonic/dystonic syndromes including patients with classic myoclonus dystonia, essential myoclonia, dystonia with myoclonus, dystonia, begin hereditary chorea. All coding exons of the SGCE gene were analyzed by direct sequencing and the DYT1 mutation was tested. We found 9 SCGE mutations (all of them novel) in 10 (29%) of the 34 patients with myoclonus dystonia, but found no mutation in any of the 50 patients with other phenotypes. There were 24 affected among 29 carriers. Penetrance was 100% in paternal and 0% in maternal transmissions. Myoclonus-dystonia patients with SGCE mutation have a younger age of onset than the non-carriers, while the two groups do not differ significantly in term of site of onset, disease progression or alcohol responsiveness. In none of our patients, psychiatric disorders were described. A single patient with myoclonus-dystonia carried the DYT1 mutation. In this large cohort of patients, we show that SGCE mutations are exclusively found in patients with typical myoclonus-dystonia but only in a subset of them (29%). Our data suggest that SGCE testing in other phenotypes is likely to be negative.

Assignment of a new congenital fibrosis of extraocular muscles type 3 (CFEOM3) locus, FEOM4, based on a balanced translocation t(2;13) (q37.3;q12.11) and identification of candidate genes. *P. Aubourg¹, M. Krahn², R. Bernard², K. Nguyen², O. Forzano³, V. Delague¹, M.G. Mattei¹, N. Philip², N. Lévy^{1,2}.* 1) Inserm U491, Faculté de Médecine Timone, Marseille, France; 2) Génétique Médicale, Hôpital d'enfants de la Timone, Assistance Publique, Marseille, France; 3) Service d'Ophthalmologie, Hôpital Timone, Assistance Publique, Marseille, France.

Congenital fibrosis of extra ocular muscles (CFEOM) is a rare condition characterised by congenital ptosis and external ophthalmoplegia of autosomal dominant (CFEOM1 and CFEOM3) or recessive (CFEOM2) inheritance. The manifestations are present at birth and do not progress through life. This condition is genetically and clinically heterogeneous with 3 phenotypes (CFEOM1 to 3) mapping to three loci (FEOM1-3), and the FEOM1 and FEOM2 genes have been identified as KIF21A and PHOX2A, respectively. Here, we report a three generation family in which four members present with congenital bilateral ptosis and restrictive ophthalmoplegia with limited upgaze, most consistent with the clinical diagnosis of CFEOM3. In this family, the phenotype is inherited in a dominant fashion and all affected members carry a translocation involving chromosomes 2q and 13q. In the last generation, one daughter carried the translocation in an unbalanced state and presented with CFEOM associated with mental retardation and facial dysmorphism. Molecular and FISH analyses mapped the 2q breakpoint at 2q37, within the Albright Hereditary Osteodystrophy (AHO) like syndrome critical interval; on the other side, 13q breakpoint maps in the 13q12 region, distal to the Connexin genes cluster, where, more likely, it interrupts a gene involved in the disorder. This CFEOM phenotype is likely to be associated to chromosome 13q12 region since no gene is interrupted by the breakpoint on chromosome 2, which one, moreover, lies in the AHO like syndrome critical region. We have identified several candidate transcripts, one of them, AKO54845 being interrupted by the translocation breakpoint on chromosome 13. This work defines a new CFEOM3 locus, FEOM4, and provides further evidence of the genetic heterogeneity observed in CFEOM.

The RAR-related orphan receptor A isoform 1 (*RORa1*) gene is disrupted by a balanced translocation t(4;15) (q22.3;q21.3) associated with severe obesity. J. Klar¹, B. Åsling², M. Uvsväck², A. Dellsén², C. Ström², M. Rhedin², A. Forslund³, G. Annerén¹, JF. Ludvigsson⁴, R. Nohra¹, N. Dahl¹. 1) Department of Genetics & Pathology, Uppsala University, Uppsala, Sweden; 2) AstraZeneca R&D, Mölndal, Sweden; 3) Department of Medical Sciences, Uppsala University, Sweden; 4) Department of Paediatrics, Örebro University Hospital, Örebro, Sweden.

We have characterized a family comprising a mother and two children with idiopathic and isolated obesity (BMI 41-49). The three family members carry a balanced reciprocal chromosome translocation t(4;15). A detailed characterization of the chromosomal breakpoints at chromosome 4 and 15, respectively, revealed that the translocation is almost perfectly balanced with a very short insertion/deletion. The chromosome 15 breakpoint is positioned in intron 1 of the RAR-related orphan receptor A isoform 1 (*RORa1*) gene. The chromosome 4 breakpoint is positioned 150 kb telomeric to the transcriptional start of the unc-5 homolog C (*UNC5C*) gene and 150 kb centromeric to the transcriptional start of the pyruvate dehydrogenase (lipoamide) alpha 2 (*PDHA2*) gene. A fusion gene containing the *RORa* exon 1 and *UNC5C* is created on the chromosome 4 derivative. Expression analyses reveal that the fusion gene is expressed in frame in adipocytes from the three affected individuals.

The rearrangement of the *RORa* gene predicts the loss of one isoform 1 (*RORa1*) allele. *RORa1* is implicated in the regulation of adipogenesis and lipoprotein metabolism. We hypothesize that the obesity in this family is caused by i) haploinsufficiency of *RORa1* or, ii) a gain of function mechanism mediated by the *RORa1-UNC5C* fusion gene.

Molecular Homogeneity of G6PD Deficiency In Bahrain. *N.J. Al Momen¹, S. Al Arrayed², A. Al Alawi³.* 1) Genetic laboratory , Salmaniya Medical Centre, Manama, Bahrain; 2) Genetic department, Salmaniya Medical Centre, Manama, Bahrain; 3) Pathology department, Salmaniya Medical Centre, Manama, Bahrain.

Objectives. This study aimed at investigating the molecular basis of G6PD deficiency in the Kingdom of Bahrain. Further emphasis will be presented on the genetic polymorphism at nucleotide 1311 for both normal and deficient subjects. **Methods:** DNA extraction was done for 83 G6PD-deficient subjects and 80 normal controls. A combination of PCR-RFLP and PCR-DGGE procedures were employed to uncover the sequence variations at nt 563 (C/T) (G6PD Mediterranean) and at nt 1311 (C/T) polymorphism in both subjects with deficient and normal G6PD activity. **Results:** Nearly Ninety-one percent (93/102) of the X chromosomes from G6PD deficient subjects had nt 563 (C-T) mutation (G6PD Med), whereas ~9% of the X chromosomes from G6PD deficient subjects might have other G6PD variant(s) (or normal X chromosomes in heterozygote females). Ninety-six percent (89/93) of the G6PD Med-bearing X chromosomes showed thymine (T) at nucleotide position 1311. In contrast, 70% (82/117) of the normal X chromosomes showed cytosine (C) at nucleotide position 1311, while it was thymine (T) in 30% (35/117) of the normal X chromosomes. **Conclusion.** The vast majority (91%) of X chromosomes from G6PD-deficient subjects in Bahrain are harboring nt 563 (C-T) mutation (G6PD Mediterranean). The G6PD Med variant in Bahrain is in tight linkage disequilibrium with thymine (T) at nt 1311. These data, collectively, revealed high molecular homogeneity of G6PD deficiency in Bahrain. **Recommendations.** Further studies are needed to uncover factor(s) contributing to heterogeneous phenotypic expression of the disease in Bahrain.

A novel SOD1 mutation in a patient with Brachial Amyotrophic Diplegia. *T. Sprovieri¹, P. Valentino², R. Mazzei¹, A. Patitucci¹, A. Magariello¹, R. Nisticó², D. Pirritano², A.L. Gabriele¹, C. Ungaro¹, A. Clodomiro², G. Peluso¹, M. Muglia¹, M. Zappia², F.L. Conforti¹.* 1) ISN/CNR, Mangone, Cosenza, Italy; 2) Institute of Neurology, University "Magna Graecia", Catanzaro, Italy.

Over 100 mutations have been described spreading through the entire Sod1 coding region, but also in the intronic and regulatory regions. This gene, located on chromosome 21q22, is implicated in the autosomal dominant form with late onset of the Amyotrophic Lateral Sclerosis. Until now, all the Sod1 mutations reported are associated to this pathological phenotype. In this study we describe for the first time a new Sod1 mutation in a patient with Brachial Amyotrophic Diplegia (BAD). The patient was a 77-year-old man with a 5-month history of severe, bilateral arm weakness and wasting. Family history was unremarkable for neurological disorders. Neurological examination showed a peculiar posture of the man-in-the-barrel. There was severe weakness and atrophy of both upper limb muscles, especially of shoulder girdles (MRC<3/5). Fasciculations were evident only on upper limb muscles. Nerve conduction studies showed lower amplitude compound muscle action potentials (CMAPs) in the median, ulnar and radial nerves bilaterally, normal motor and sensory nerve conduction, without significant conduction block. Needle electromyography showed denervation potentials with reduced recruitment in all upper limb muscles. A wide screening was performed to exclude other causes of man-in-the barrel syndrome. Molecular analysis of the survival motor neuron gene and X-linked spinobulbar muscular atrophy was negative. Genetic investigation of the five exons of the Sod1 gene by DHPLC and direct sequencing of the PCR products, showed a variant profile of exon 4 caused by a heterozygous CT substitution at position 1126 of the gene (Leu106Pro). This mutation was not found in more than 150 control subjects from southern Italy. In conclusion, Sod1 mutations have been actually described in a small percentage of apparently sporadic cases of ALS. Our patient is the first case of sporadic lower motoneuron disease with a mutation in the SOD1 gene. The present findings suggest that Sod1 gene should be investigated in subject with BAD.

***SALL4* deletions are a common cause of Okihiro and acro-renal-ocular syndromes and confirm**

haploinsufficiency as the pathogenetic mechanism. W. Borozdin¹, D. Boehm², M. Leipoldt¹, C. Wilhelm¹, W. Reardon³, J. Clayton-Smith⁴, K. Becker⁵, H. Muehlendyck⁶, R. Winter⁷, O. Giray⁸, F. Silan⁹, J. Kohlhase¹. 1) Inst. for Human Genetics, Univ. of Freiburg, Germany; 2) Inst. for Human Genetics, Univ. of Göttingen, Germany; 3) Natl. Centre for Medical Genetics, Dublin, Ireland; 4) Dept. of Clin. Genetics, St Marys Hospital, Manchester, UK; 5) Kennedy-Galton Centre, Harrow, UK; 6) Dept. of Neuroophthalmology, Univ. of Göttingen, Germany; 7) Inst. for Child Health, London, UK; 8) Dept. of Pediatrics, Genetics Div., Dokuz Eylul University, Izmir, Turkey; 9) Med. Biology and Genetics Dept., Abant Izzet Baysal University, Duzce, Turkey.

Okihiro (Duane Radial-Ray) syndrome is an autosomal dominantly inherited malformation syndrome characterized by radial malformations of upper limbs in combination with Duane anomaly. Previously, mutations in the putative zinc finger transcription factor gene *SALL4* were reported to cause Okihiro and acro-renal-ocular syndrome. However, in a substantial proportion of patients with such phenotypes no *SALL4* mutation was found. Segregation analysis of five intragenic SNPs was informative in four out of six families and suggested a heterozygous *SALL4* deletion of at least exon 2. To confirm these findings and establish the deletion size, quantitative real time PCR was applied. In this way, we identified two families with deletions of all four exons as well as two families with deletions of exons 1-3. In one family uninformative for the five intragenic SNPs, a heterozygous deletion of exon 4 was detected, and in a further family, a heterozygous deletion of exon 1 was found. In the latter, a deletion size of 8.9 kb was determined with one breakpoint residing within an Alu-element. In another family, the deletion size was 59.4 kb with both breakpoints in Alu-elements, suggesting that Alu-mediated recombination is responsible for at least some of the deletions. These results show that, in contrast to the likely dominant-negative action of *SALL1* mutations causing Townes-Brocks syndrome, Okihiro and acro-renal-ocular syndromes are clearly resulting from *SALL4* haploinsufficiency.

Common 5q35 microdeletions in Sotos syndrome result from DNA rearrangements between directly oriented subunits of large low copy repeats. *N. Kurotaki¹, K. Wakui², J.-F. Cheng³, S.A. Yatsenko¹, N. Matsumoto⁴, P.*

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Sotos syndrome (SoS) is a well-characterized overgrowth syndrome with mental retardation, specific craniofacial features, and advanced bone age. Since NSD1 haploinsufficiency was determined to be the major cause of the syndrome, many intragenic mutations and chromosomal microdeletions involving the entire NSD1 gene have been described. In the Japanese population, approximately half of patients with SoS has a common microdeletion. We have analyzed DNA sequence of genomic segments flanking SoS common deletion. BLAST search showed 3 unique repeat sequences, termed Proximal Sos-REP (390 kb), Distal Sos-REP-A (326 kb), and Distal Sos-REP-B (50 kb). We determined the size, orientation, and extent of homology of these repeat sequences. Blast comparison of Proximal- Sos-REP and Distal Sos-REP-A revealed five subunits which were numbered from subunit 1 to 5. Each subunit was in an inverted orientation although the order of subunits was different between the two REPs. In contrast, Distal Sos-REP-B that was more than 50 kb telomeric of Distal Sos-REP-A consists of only directly oriented subunit 2. Distal Sos-REP-B and subunit 2 in other REPs are more than 98% identical. Using pulsed-field gel electrophoresis analysis, we detected a novel 0.4 Mb junction fragment in seven SoS patients with common deletion. This suggests that both Proximal- Sos-REP and Distal Sos-REP-B act as a homologous recombination substrate for the Sotos syndrome common microdeletions.

Embryonic expression and mutation analysis of the Cornelia de Lange syndrome gene, NIPBL. *E.T. Tonkin¹, T-J. Wang¹, S.N. Lisgo¹, M. Ireland¹, M. Krajewska-Walasek², T. Strachan¹.* 1) Inst Human Genetics, Univ Newcastle, Newcastle, United Kingdom; 2) Department of Medical Genetics, Children's Memorial Health Institute, Warsaw, Poland.

We recently reported identification and preliminary characterisation of NIPBL, a novel gene mapping to 5p13.1 that we showed to be mutated in Cornelia de Lange syndrome (Tonkin et al., 2004). The NIPBL gene has 46 coding exons and specifies a protein, delangin, that is significantly related to the fruitfly developmental regulator Nipped-B, and to fungal Scc2-type proteins that perform basic chromosomal functions, notably sister chromatid cohesion. We now report extended mutation analyses that survey all 46 coding exons. As well as exploring the molecular basis of phenotype discordance in sibs, our mutation analyses provide insights into the molecular basis of classical and mild CdLS. We also report extended embryonic expression analyses, including novel detailed studies of expression in the developing brain and limbs, and in craniofacial and genito-urinary systems. The pattern of embryonic expression is largely consistent with that expected from pathogenesis, but unanticipated expression patterns in some systems may provide new insights into the pathogenesis. We also present an evolutionary analysis that relates human delangin to novel vertebrate, nematode and plant counterparts as well as to known fungal and fruitfly homologues.

Fine mapping of the X-linked split-hand/split-foot malformation locus and mutation analysis of candidate genes.

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Split-hand/split-foot malformation (SHFM) is a genetically heterogeneous inherited disorder, in which affected individuals display lack of median digital rays, syndactyly, and aplasia/hypoplasia of the phalanges, metacarpals and metatarsals. Four autosomal and a single X-linked locus have been reported. In the X-linked SHFM family we have previously reported linkage to a 22 Mb interval on Xq24-q26. To fine map this region and to obtain additional phenotypic details of the heterozygous carrier females, the SHFM index family was revisited. In this family, all 39 affected hemizygous males and 3 homozygous females exhibited the trait while fifteen of the obligate heterozygous females showed partial and asymmetric expression of SHFM. Analysis of DNA from 37 individuals, including 21 males and 7 females who partially or fully expressed the SHFM trait, exposed a new centromeric boundary at DXS1114 while the telomeric boundary was defined by the DXS1192. These data further refined the SHFM2 locus to a 5.1Mb region. Several candidate genes including TDU, ZIC3, FGF13 and FHL1, which are expressed in developing limb, reside within the mapped region. No mutations were identified in the coding regions of these functional and fifteen of the positional candidate genes. These data suggest that the mutation may lie in a regulatory region of one of these candidate genes or in another gene within the SHFM2 locus with unclear role in limb development.

Haplotype-specific BAC libraries for identification of dominant amyotrophic lateral sclerosis (ALS) genes on 16q12 and 18q21. *Y. Yoshinaga*¹, *T.J. Kwiatkowski, Jr*², *F. Gros-Louis*³, *C. Gros-Louis*⁴, *M. Nefedov*¹, *K. Osoegawa*¹, *J.S. de Belleruche*⁵, *G.A. Rouleau*³, *R.H. Brown, Jr*², *P.J. de Jong*¹. 1) BACPAC Resources, Children's Hospital Oakland, Oakland, CA; 2) Harvard Medical School, Charlestown, MA; 3) Montreal General Hospital Research Institute, Montreal, Qc, Canada; 4) Broad Institute, Cambridge, MA; 5) Imperial College London, London, UK.

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by a selective loss of motor neurons. Approximately 10% of cases are familial (FALS), mostly in an autosomal dominant form. While 25% of the familial cases are caused by mutations in the Cu/Zn superoxide dismutase gene (SOD1), the remaining familial cases are predisposed due to dominant FALS loci on a variety of other chromosomes, e.g. recently mapped ALS6 on 16q12 and ALS3 on 18q21. Our strategy to identify these ALS genes is to complete the high-quality sequencing of the affected haplotype by sequencing the haplotype-specific BAC contigs. The diploid-to-haploid conversion method was employed to create human-murine hybrid cells harboring only the haplotype linked to the human disease-allele-bearing chromosome. Three >500,000-clone libraries have been prepared from hybrid cell lines from two patients containing the 16q12 and one patient containing the 18q21 FALS loci. Each library was spotted on 28 high-density colony filters and screened using very complex probes pools (~150/pool), which are designed at regular (50 kbp) intervals along the affected 4-7 Mbp regions. All positive clones were BAC-end sequenced to prepare a BAC-contig relative to the assembled human genome containing with less than 2% of the sequence not present in the contigs. A minimum set of BAC clones for the affected regions have been processed by the shotgun sequencing. The BACs will also be used as reagents to create surrogate phenotypes as a means to narrow the choice of candidate genes. We are further optimizing haplotype-specific BAC construction protocols to test whether this approach can be applied in the search for additional dominant gene mutations. Our research is supported in part by a grant from the ALS Association.

Male-to-male transmission in X-linked Alport Syndrome. *E. Ars*¹, *B. Tazón-Vega*¹, *P. Ruiz*¹, *C. Nogués*², *O. Rajmil*³, *R. Torra*⁴. 1) Molecular Biology Laboratory, Fundació Puigvert; 2) Universitat Autònoma Barcelona; 3) Andrology Dept., Fundació Puigvert; 4) Nephrology Dept., Fundació Puigvert. Barcelona. Catalonia. Spain.

Alport Syndrome (AS) is a hereditary nephropathy affecting the glomerular basement membrane often associated with hearing loss. Genetically the disorder is heterogeneous and can be transmitted following an X-linked or an autosomal pattern of inheritance. X-linked AS is the most frequent form of the disease and is caused by mutations in the COL4A5 gene at Xq22-q23, while the autosomal form is due to mutations in the COL4A3 and COL4A4 genes, both located on chromosome 2q36-q37. Male-to-male transmission has been considered fully indicative of autosomal AS. Here we report a family with male-to-male transmission in which linkage to the COL4A3 and COL4A4 genes was excluded. Unexpectedly, linkage to chromosome X was demonstrated and it identified an extra X chromosome of paternal origin in the proband. Cytogenetically, the proband 47,XXY karyotype was confirmed and analysis of paternal sperm did not show an abnormal percentage of diploid spermatozoa. Moreover, a splicing mutation was characterized by analysis of the whole coding region of the COL4A5 gene. The outcome of this patient is unpredictable but the presence of microhematuria and proteinuria at 4 years of age reminds more of an affected boy than a female carrier. To our knowledge this is the first reported case of concurrence of Alport and Klinefelter syndromes. Bearing in mind that their incidences are 1 in 5,000 and 1 in 1,000 male births, respectively, the probability for the simultaneous occurrence of these two disorders is approximately 1 in 5,000,000 births. In spite of the unlikelihood of this phenomenon it should be considered when offering genetic counseling for AS families.

Elucidation of pathogenic genetic pathways in the hypertrophic heart using microarray technology. *H. Smeets¹, B. Van den Bosch¹, P. Lindsey¹, D. Lips², C. Van den Burg¹, P. Doevendans², R. Vlietinck¹.* 1) Dept of Genetics & Cell Biol, Univ Maastricht, Maastricht, Netherlands; 2) Dept of Cardiology of the Heart Lung Centre Utrecht (UMCU), Utrecht, Netherlands.

Introduction Gene expression differences between normal, hypertrophic and failing heart may unravel underlying pathogenic mechanisms in patients. To circumvent the complexity and scarcity of human biopsies, we used the Transverse Aortic Constricted (TAC) mouse model for cardiac hypertrophy. By gene expression profiling we aim at identifying the molecular program underlying myocardial hypertrophy, the most powerful predictor of heart failure. **Methods** Male Swiss mice (10 weeks old) were either TAC or sham-operated (n=5) and sacrificed at 48 hours, 1 week, 6 or 8 weeks after surgery. Hearts were harvested and hypertrophy was assessed by determining the ratio left ventricle weight/tibia length. Left ventricle RNA was analysed on microarrays containing 15,000 embryonic and fetal cDNA inserts (National Institute on Aging) and 4,200 controls, including RNA spikes, housekeeping genes and negative controls. **Results** At 48 hours, 1 week, 6 and 8 weeks after banding, respectively 779, 537, 1303 and 621 genes were significantly differentially expressed with fold changes ranging from 1.1 till more than 15. The differential genes included hypertrophy markers, like MHC and SERCA2, genes in known hypertrophic processes, like MAPK pathways and genes in calcium-signaling pathways. During the early hypertrophic phase, most genes were up-regulated, while at a later stage genes were mostly down-regulated. Early identified processes involved mainly signalling and transcription related genes, while energy metabolism and cell adhesion were mainly involved at a later stage. **Conclusions** Using a well-defined array design and data analysis we were able to identify significantly small changes in gene expression for large numbers of genes. Known genes and markers confirmed the hypertrophy process occurring. More extensive study of the data will be presented with new pathways and genes involved in the process of cardiac hypertrophy and may unravel targets for interventions.

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***MutationView* : Development of an Intelligent Search System to Extract Disease-associated Knowledge. M.**

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In order to analyze the relation between diseases and genetic diversity, we developed an integrated database system *MutationView*, which at present mainly deals with monogenic diseases (<http://mutview.dmb.med.keio.ac.jp>).

MutationView can search, display and analyze the mutation data with graphical environment. The characteristic features of the *MutationView* include : (1) Various data display and analysis functions are available with regard to genomic/cDNA structure, functional domains of protein, the histogram of the case number of each mutation, changes in the nucleotide sequence and restriction sites, and the symptom associated with each mutation. (2) Mutation data are collected on the diseases related to eye, ear, brain, muscle, heart, autoimmunity and familial tumor.

To date, we have collected 9762 entries of mutations from 1706 literatures dealing with 251 genes involved in 235 distinct diseases. A new category systemic bone diseases including osteochondrodysplasia has been added for 31 genes. For more effective use of the *MutationView*, we have developed a new support system to automatically extract disease-associated knowledge from the literature. In addition to categorized dictionary for various fields such as clinical medicine, histology and anatomy, we have created an automatic extraction method of keywords based on the frequency of word usage. Using these, the OMIM documents are now in process of statistical language analysis. This knowledge extraction system will be implemented to *MutationView*. Computer demonstration will be performed at the meeting.

Phenotypic and Molecular Characterization of Patients with BOR Syndrome: Mutation Screening of EYA1 and SIX1 Genes and Issue of Genetic Heterogeneity. *U. Radhakrishna¹, C. Holley¹, F. Hildebrandt², C.W.R.J. Cremers³, W.J. Kimberling¹, S. Kumar¹.* 1) Dept. of Genetics, Boys Town National Res. Hosp., Omaha, NE; 2) Department of Pediatrics, University of Michigan, Ann Arbor, MI; 3) Department of Otolaryngology, University Hospital Nijmegen, The Netherlands.

Branchio-oto renal syndrome (BOR) is a human developmental disorder (autosomal dominant) which produces a wide range of clinical manifestations such as (1) branchial cleft, fistulas or cysts (2) various ear anomalies such as malformed pinnae, pre-auricular pits, structural defects of the external, middle and/or inner ear (3) hearing loss and (4) renal anomalies. It affects at least 2% of profoundly deaf children and have estimated prevalence of 1 in 40,000.

The first BOR gene has been localized to chromosome 8q13 and EYA1 gene has been implicated. We have performed mutation analysis on more than eighty BOR families by hetero-duplex followed by sequence analysis of sixteen EYA1 exons. At least 55% of our large data set of BOR families do not show mutations in the EYA1 gene. This further complicates the issue. A second genetic locus was mapped to 1q and recently, third locus was identified on chromosome 14q. Within the critical region of 14q, SIX1, SIX4 and SIX6 candidate genes have been located, which act within a genetic network of EYA and PAX genes to regulate organogenesis. Using direct sequencing, we identified mutations with SIX1 gene in BOR/BO kindreds confirming SIX1 as a new gene involved in branchiogenetic disorders. The present results, together with mutation screening and genetic linkage study, demonstrate genetic heterogeneity. A genome-wide search is being performed for the localization of fourth locus. Also, a candidate gene analysis is underway to identify genes in the critical region of second BOR locus on chromosome 1q.

These results provide the basis for a molecular-genetic testing that will help the clinical evaluation and genetic counseling of members of BOR families. Further characterization of EYA1 and SIX1 mutation and identification of other BOR genes will significantly help in defining the spectrum of defects associated with branchial and hearing anomalies.

Reduced penetrance of craniofacial anomalies as a function of deletion size and genetic background in a chromosome engineered partial mouse model for Smith-Magenis syndrome. *J. Yan*¹, *V.W. Keener*¹, *W. Bi*¹, *K. Walz*¹, *A. Bradley*³, *M.J. Justice*¹, *J.R. Lupski*^{1,2}. 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Texas Children's hospital, Houston, TX; 3) The Wellcome Trust Sanger Institute, Hinxton, UK.

Smith-Magenis syndrome (SMS) is a multiple congenital anomaly/mental retardation syndrome associated with del(17)(p11.2p11.2). The phenotype is variable even in patients with deletion of the same size. *Df(11)17/+* mice contain a heterozygous deletion in the mouse region syntenic to the SMS common deletion, and exhibit craniofacial abnormalities, seizures, and marked obesity, partially reproducing the SMS phenotype. To narrow the genomic interval responsible for the phenotype, we constructed 3 lines of mice with smaller deletions (*Df(11)17-1*, *Df(11)17-2*, and *Df(11)17-3*) using retrovirus mediated chromosome engineering to create nested deletion. Both craniofacial abnormalities and obesity have been observed, but the penetrance of the craniofacial phenotype is markedly reduced in comparison to *Df(11)17/+* mice. Overt seizures were not observed. Phenotypic variation has been observed in mice with the same deletion size in both the same and different genetic backgrounds, which may reflect the variation documented in the patients. These results indicate that the smaller deletions contain the genes for the craniofacial abnormalities and obesity. However, genes or regulatory elements in the larger deletion that are not located in the smaller deletions, as well as genes located elsewhere, also influence both the penetrance and expressivity of the phenotype. Our mouse models refined the genomic region important for a portion of the SMS phenotype and provide a basis for further molecular analysis of genes associated with SMS.

Activating mutations in *KCNJ11* cause transient as well as permanent neonatal diabetes with functional severity of activating mutation determining phenotype. A.L. Gloyn^{1,2}, F. Reimann³, C. Girard¹, E.L. Edghill², E.R. Pearson², D.J.G. Mackay⁴, I.K. Temple⁴, J.P.H. Shield⁵, K. Noyes⁶, D. Freedenberg⁷, S. Ellard², F.M. Ashcroft¹, F.M. Gribble³, A.T. Hattersley². 1) University of Oxford, UK; 2) Peninsula Medical School, Exeter, UK; 3) University of Cambridge, UK; 4) Southampton, UK; 5) Bristol, UK; 6) Edinburgh, UK; 7) Austin, Texas, USA.

The ATP-sensitive potassium (K_{ATP}) channel is a key candidate gene for diabetes, it couples metabolism to membrane electrical events and insulin secretion, with ATP closing the channel. We have shown that heterozygous activating mutations in *KCNJ11*, which encodes the Kir6.2 subunit of the β -cell K_{ATP} channel, are a common cause of permanent neonatal diabetes (PNDM) and common genetic variation in *KCNJ11* (E23K) predisposes to type 2 diabetes (T2DM). Transient neonatal diabetes (TNDM) is a rare type of diabetes that presents soon after birth and resolves by 18 months. We hypothesised that an array of naturally occurring *KCNJ11* activating mutations could exist with a range of functional severities resulting in diabetes with a spectrum of clinical presentations. Therefore we investigated the role of *KCNJ11* mutations in TNDM. *KCNJ11* was sequenced in 11 probands with TNDM. Identified mutations were expressed in *Xenopus* oocytes and functionally characterised by patch clamp analysis. Novel heterozygous mutations (G53S, G53R, I182V) which co-segregated with diabetes were identified in 3 probands. Mutations were not identified in 100 normal chromosomes. Functional analysis of the G53S and I182V mutations demonstrated that they resulted in a decrease in affinity for ATP, (K_i ; WT 8.9 2.1 vs G53S 37.8 7.6, $p = 0.0018$, I182V 28.9 3.3, $p < 0.001$, $n=10$). These defects are functionally less severe than for the R201H PNDM mutation, which had a 40 fold reduction in ATP sensitivity. Our study demonstrates a spectrum of association of *KCNJ11* with diabetes with severe mutations presenting as PNDM and milder mutations presenting as TNDM and a common polymorphism predisposing to T2DM. These results may have implications for the clinical management of these patients.

Searching for a novel MODY gene in a large Norwegian family with autosomal dominant diabetes. S.

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Background and aims: Maturity-onset diabetes of the young (MODY) is a heterogeneous subgroup of diabetes with autosomal dominant mode of inheritance and beta cell dysfunction. Approximately 20% of families fitting MODY criteria do not have mutations in any of the six genes where mutations are known to cause MODY. We have investigated a large Norwegian family with autosomal dominant diabetes. The aim was to map a possible novel MODY locus. Subjects and methods: We studied 58 family members. Fourteen subjects had diabetes associated with a primary beta cell dysfunction and one had impaired glucose tolerance. Candidate genes were investigated either by direct sequencing in selected affected individuals or by linkage analyses using microsatellite markers flanking the locus. Results: Median age of diabetes diagnosis was 38 years (range 18-56 years) and eight of the diabetic patients were on insulin therapy. Mean BMI among diabetes patients was 24.4 kg/m² and mean fasting C-peptide was 0.30 nmol/l. Markers of autoimmunity were negative. No mutations were detected in the six known MODY genes. Furthermore, three genes encoding transcriptional factors involved in pancreas development and differentiation and five genes with a role in regulation of glucose metabolism were also excluded. The affected family members all had at least one HLA-DQB1*0602 allele known to be highly protective against autoimmune type 1 diabetes, but linkage and haplotype analyses excluded the HLA complex as the disease locus. Conclusion: We have studied a large family with monogenic diabetes fitting clinical MODY criteria. The disorder is probably caused by mutation in a novel MODY gene. Simulations of pedigree data indicate that this family may be suitable for a whole genome linkage screen. We have therefore initiated such a screen to detect the postulated novel MODY locus.

Identification of a novel mutation of the Kir6.2 gene in a Japanese family with dominantly inherited diabetes mellitus. *T. Yorifuji, K. Kurokawa, M. Mamada, M. Kawai, T. Nakahata.* Dept Pediatrics, Kyoto Univ Hosp, Kyoto, Japan.

Dominantly inherited diabetes mellitus with decreased insulin secretion is typically known as MODYs (Maturity Onset Diabetes of the Young). So far, 6 subtypes of MODYs, MODY1-6, are known with mutations in different genes. In Japan, however, MODY1-6 comprise a relatively small fraction of patients with dominantly inherited diabetes mellitus. In this study, we performed a multipoint linkage analysis of a four-generation family with dominantly inherited diabetes mellitus. The onset of diabetes varied among family members, the earliest one presented in the neonatal period and others presented later in life. Several regions of interest were identified and compared with locations of previously known genes related to insulin secretion or pancreatogenesis. By sequencing analysis of the candidate genes, we identified a novel missense mutation (C42R) in the Kir6.2 gene. Currently we are in the process of functional analysis of this mutation. If this is causing diabetes mellitus in this family, it follows that mutations in Kir6.2 could be a cause of not only neonatal diabetes but also of later onset diabetes mellitus.

A mutation in bovine keratin 5 causing epidermolysis bullosa simplex, transmitted by a mosaic sire. *H.E. Ward¹, C.A. Ford¹, A.M. Stanfield¹, A. Walden¹, M. Al-Wahb¹, E. Bohm¹, G.T. Sutherland¹, B. Smits², H. Holloway³, A.E.L. Ankersmit-Udy⁴, K. Cottier⁴, R.J. Spelman⁴, R.G. Snell¹.* 1) Bovine Genetics, ViaLactia Biosciences, Auckland, New Zealand; 2) Alpha Scientific Ltd, Hamilton, New Zealand; 3) University of Auckland, Auckland, New Zealand; 4) Livestock Improvement Corporation, Hamilton, New Zealand.

A mechanobullous skin disorder was reported in progeny of a three year-old Friesian-Jersey crossbred bull. The condition presented clinically as the loss of skin and mucosa from contact areas and resultant inflammation. Examination of skin samples from affected animals under light microscopy revealed separation of the epidermis from the underlying dermis. Electron microscopic analysis refined the site of cleavage to above the basement membrane and involving lysis of basal keratinocytes. These observations were consistent with the simplex form of epidermolysis bullosa (EB simplex) in humans. Potential candidate genes based on known human causative gene mutations were assessed, resulting in keratin 5 being identified as the most likely mutation-bearing gene. The sequence of bovine keratin 5 was established and direct sequencing led to identification of a G to A substitution common in all the affected animals. This mutation leads to an amino acid change of glutamic acid to lysine in the final E (478) of the KLLEGE motif of the protein. The sire carried a de novo mutation and was mosaic, explaining his asymptomatic status and the less than expected frequency of affected offspring. Remarkably, the same mutation has been previously described in EB simplex in humans. Semen has been collected from the bull, which could be used to generate a model for human EB simplex.

Mis-expression of the *Drosophila melanogaster* orthologue of human DMC1 disrupts the hedgehog signaling pathway in the fly wing. L. Hull¹, D.H. Cohn², L. King², L. Reiter¹, E. Bier¹. 1) Department of Biology, UC San Diego, La Jolla, CA; 2) Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA.

Dyggve-Melchior-Clausen dysplasia (DMC) and Smith-McCort dysplasia (SMC) are recessive allelic disorders that result from mutations in the DMC1 gene. Homozygosity or compound heterozygosity for null mutations causes DMC, a syndrome characterized by short stature and mental retardation. Compound heterozygosity for a missense mutation and an exon-skipping mutation in the gene have been found in two families with SMC, which present the same skeletal defects but lack the psychomotor dysfunction found in patients with DMC. Bioinformatic searches based on the sequence of the encoded protein, *dymeclin*, have uncovered putative transmembrane segments but no homology to any known functional protein domains. Thus function of the protein and the mechanism by which the mutations produce the phenotype are unknown. We have employed the model organism, *Drosophila melanogaster*, to uncover the function of the ubiquitously expressed protein *dymeclin*. Since *dymeclin* is highly conserved from *D. melanogaster* to humans, we used the GAL4-UAS system in *Drosophila* to overexpress human *dymeclin* as well as its unique *Drosophila* ortholog (dDMC) in a tissue specific manner. Misexpression of dDMC at high levels in the wing reduced the overall size of the wing, and at lower levels, selectively reduced the intervein space between wing veins L3 and L4. This milder phenotype was similar to the phenotypes produced by inhibition of Hh signaling found in either Hh mutants or by overexpression of the inhibitory components of the Hh signaling pathway. These data suggest that *dymeclin* overexpression disrupts hedgehog (Hh) signaling in the A/P organizer of the wing. Overexpressing dDMC in only the posterior portion of the wing did not affect L3-L4 intervein development, indicating that Hh synthesis was unaffected by increased *dymeclin*. Anterior overexpression, however, again resulted in a decrease in the distance between L3 and L4, indicating that *dymeclin* overexpression inhibits Hh signaling in cells receiving the Hh signal in the developing *Drosophila* wing.

IVS8-1G>C and T93M are the most frequent 7-dehydrocholesterol reductase mutations identified in a cohort of Smith-Lemli-Opitz syndrome patients from Brazil. *F.B. Scalco¹, L.S. Correa-Cerro¹, C.A. Wassif¹, D. Moretti-Ferreira², F.D. Porter¹.* 1) NICHD, National Institutes of Health, Bethesda, MD; 2) Servico de Aconselhamento Genetico, UNESP, Botucatu, SP, Brazil.

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive inborn error of cholesterol biosynthesis, caused by mutation of the 7-dehydrocholesterol reductase gene (*DHCR7*). *DHCR7* reduces 7-dehydrocholesterol (7DHC) to yield cholesterol in the final step of the cholesterol biosynthetic pathway. Biochemically, SLOS patients have elevated serum and tissue 7DHC levels and typically have low cholesterol levels. Over one hundred mutant *DHCR7* alleles have been identified. The most frequently reported *DHCR7* mutant allele is IVS8-1G>C. IVS8-1G>C is a splice acceptor mutation which causes aberrant splicing which results in the incorporation of 134 nucleotides of intronic sequence into the *DHCR7* mRNA. IVS8-1G>C is a null mutation. The second most frequently reported *DHCR7* mutant allele is T93M (278 C>T). *DHCR7*^{T93M} has been reported as being a common mutation in Italian and Cuban patients. We performed *DHCR7* mutation analysis for 14 biochemically confirmed SLOS patients and their parents from South and Southeastern Brazil. For this study, we performed mutation analyses by sequencing exons 3-9 of the *DHCR7* gene and SNP genotyping for IVS8-1G>C. The most frequent mutations found in this cohort were IVS8-1G>C (10/28 alleles) and T93M (9/28 alleles). One infant was homozygous for IVS8-1G>C. In six patients, the *DHCR7* genotype was T93M/IVS8-1G>C. Haplotype analysis was performed, and in this group of patients, the T93M mutation was found in association with haplotype L. Previously, the T93M mutation has been identified on haplotypes A, J, and K. This new and consistent association of the T93M mutation with haplotype L suggests a founder effect for this mutant allele in this Brazilian population.

Winchester Syndrome is caused by mutations in the matrix metalloproteinase 2 gene (MMP2). A. Superti-Furga¹, V. Calcaterra², M. Di Rocco³, A. Zankl¹. 1) Mol Ped Div, University of Lausanne, Lausanne, Switzerland; 2) Department of Pediatrics, University of Pavia, Pavia, Italy; 3) Pediatric Unit, Istituto Gaslini, Genoa, Italy.

The inherited osteolysis syndromes are a heterogeneous group of skeletal disorders whose classification is still uncertain. Three osteolysis syndromes show autosomal recessive inheritance and multicentric involvement predominantly of the hands and feet: Torg syndrome (OMIM 259600), Winchester syndrome (OMIM 277950), and Nodulosis-Arthropathy-Osteolysis syndrome (NAO, OMIM 605156). The latest Nosology of the International Skeletal Dysplasia Society, classifies NAO as a variant of Torg syndrome, while Winchester syndrome is considered a separate disorder. The main differences between Torg/NAO and Winchester syndrome are the presence of subcutaneous nodules in the former and more severe osteolysis and variable skin findings (but no nodules) in the latter. Recently, mutations in the matrix metalloproteinase 2 (MMP2) were reported in 2 families with prominent subcutaneous nodules, moderate osteolysis and arthropathy and a clinical diagnosis of NAO. We identified a homozygous missense mutation in MMP2 in a 19-year old girl with typical features of Winchester syndrome: severe childhood-onset generalized osteolysis, short stature, mildly coarsened facial features and hypertrichosis, but no subcutaneous nodules. Additional findings included type I diabetes mellitus and hypothyroidism. The mutation (E404K) substitutes an amino acid believed to have a key role in the catalytic activity of MMP2 and is therefore likely to cause complete loss of MMP2 function in the homozygous state. Molecular and clinical findings in this case indicate that Torg/NAO and Winchester syndrome are allelic disorders and represent a clinical spectrum that remains to be explored.

Mutations in the C-propeptide of COL2A1 cause platyspondylic lethal skeletal dysplasia, Torrance type. A.

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Platyspondylic lethal skeletal dysplasia Torrance type (PLSD-T) is a rare chondrodysplasia characterized by varying platyspondyly, brachydactyly and metaphyseal changes. Recently, mutations in the C-propeptide of type II collagen have been identified in two patients with PLSD-T, suggesting that PLSD-T may be a type II collagen-associated disorder. We present 8 additional cases with PLSD-T and mutations in the C-propeptide domain of COL2A1. The mutational spectrum includes missense, stop codon and frameshift mutations. All nonsense mutations are located in the last exon, where they could escape nonsense-mediated mRNA decay. We propose that PLSD-T is specifically caused by mutations in the COL2A1 C-propeptide domain, which lead to biosynthesis of an altered collagen chain (as opposed to a null allele). The phenotypic effects could result from a combination of diminished collagen fibril formation, toxic effects through the accumulation of mutated collagen chains inside the chondrocytes and alteration of a putative signaling function of the C-propeptide of type II collagen. We have recently suggested a similar model for spondyloperipheral dysplasia, whose clinical and radiographic features are comparable to those of long-term survivors with PLSD-T (Zankl et al., *Am J Med Genet*, in press). We propose that spondyloperipheral dysplasia and PLSD-T constitute a subfamily of the type II collagenopathies, characterized by distinctive radiological features and specific mutations in the C-propeptide domain.

The reproducibility of mRNA expression and immunoreactive levels of FSH in Chinese hamster ovary cells stably transfected with wildtype and mutant Ala43Pro human FSHB gene constructs. *L.C. Layman, A.D. Clark.* Section of Reproductive Endocrinology, Infertility, & Genetics, Dept OB/GYN, Program in Developmental Neurobiology, Institute of Molecular Medicine, & Genetics, Medical College of Georgia, Augusta, GA.

Mutations in the human follicle stimulating hormone-beta (FSHB) gene cause absent or severely impaired puberty and infertility. All mutations to date result in mRNA expression, but absent protein levels in vitro. Although controls are always used in these assays, the variability of different stably transfected cell lines is unknown, even when colonies are derived from the original plate of cells transfected. The purpose of the current study was to: 1) determine the variability of FSHB expression and immunoreactive FSH levels of individual clones derived from the original plate of transfected cells; and 2) determine if a HTZ Ala43Pro FSHB mutation identified in a female with premature ovarian failure affects transcription and protein levels in vitro. Site directed mutagenesis of pFSH constructs was performed, followed by stable transfection in Chinese hamster ovary (CHO) cells using antibiotic resistance. Either the WT alone, Ala43Pro mutant alone, or both the mutant and WT were stably transfected into CHO cells. Real-time RT-PCR and immunoassay for FSH were then performed. FSH levels were markedly elevated in the cell line expressing Ala43Pro. The initial expression results revealed no difference among the three groups (n= 4 clones each), with mean + SEM as follows: WT=12.17 + 3.89 fg/uL; Ala43Pro = 19.01 +11.90; WT/Ala43Pro = 22.62 +9.65. There was a trend toward increased expression for cell lines coexpressed with WT and Ala43Pro. Additional clones are being analyzed to determine if these preliminary findings hold true with an adequate sample size, as determined by power analysis. In contrast to all previously reported FSHB mutations, the Ala43Pro mutation results in increased immunoreactive FSH levels in vitro, which could be due to increased expression.

Spontaneous mutation over the lifespan of the mouse: Low and constant mutation frequency in the male germline and unchanged mutation types with aging. K.A. Hill^{1,2}, K. Farwell¹, J. Longmate³, W.A. Scaringe¹, J. Wang¹, S.S. Sommer¹. 1) Dept of Molecular Genetics, City of Hope Ntnl Med Ctr, Duarte, CA 91010; 2) Dept of Biology, Univ of Western Ontario, London, ON Canada N6A 5B7; 3) Division of Information Sciences, City of Hope, Ntnl Med Ctr, Duarte, CA 91010.

The frequency and pattern of spontaneous mutation was determined in individual tissues over the life cycle of Big Blue mice. A total of 95.6 million *lacI* transgene sequences were screened, 2,995 mutant transgenes harvested and 2,428 independent mutations identified. Detailed analyses included seven to nine time points during the lifespan of the mouse in adipose tissue and male germline from adolescence/young adulthood (3 months) to very old age (30 months) and in liver and neurons from pre-weaning (10 days) to very old age. The data reveal at least two tissue-specific profiles of spontaneous mutation frequency (elevation with age in adipose tissue and liver and constancy in neurons and male germ cells) and a low mutation frequency in the male germline. Despite differences in the profiles of spontaneous mutation frequency with age, mutation patterns are unchanged with age within a tissue. In middle to late adulthood, significant deviations in the mutation pattern are observed *among* the four tissues, consistent with a minor effect of tissue-specific metabolism. There are no obvious correlations between the rate of cell proliferation and mutation frequency profile with age or deviations in the mutation pattern. The absence of changes in the pattern of most mutations with age despite large numbers of mutations suggests that the qualitative nature of DNA damage and repair does not change with age. Unaltered balance of DNA damage and repair with age in the germline has evolutionary consequences and is of particular interest given the controversy over whether or not increasing germline mutation frequency with paternal age is the cause of reports associating older males with a higher incidence of some types of genetic disease. These most detailed measurements help to constrain hypotheses regarding the role of mutational mechanisms in DNA repair and aging.

Molecular relationships between Myotonic Dystrophy type 1 and 2 pathogenesis. A. Botta¹, L. Vallo¹, E. Bonifazi¹, S. Caldarola², D. Fruci³, A. Nardone¹, F. Gullotta¹, R. Massa⁴, F. Loreni², G. Novelli¹. 1) Dept Biopat, Tor Vergata Univ, Rome, Italy; 2) Dept Biology, Tor Vergata Univ, Rome, Italy; 3) Res Center, Osp Bambino Gesù', Rome, Italy; 4) Dept Neurosc, Tor Vergata Univ, Rome, Italy.

Myotonic Dystrophy (DM) is caused by two mutations: a CTG expansion in 3'UTR region of the DMPK gene (DM1; OMIM 160900) and a CCTG expansion in intron 1 of the ZNF9 gene (DM2; OMIM 602688) respectively on chromosome 19p13 and 3q21. The most accredited mechanism for DM pathogenesis is an RNA gain-of-function. Other findings suggest at least a contributory role of the of DMPK-insufficiency and, by analogy, an altered expression of ZNF9 gene and/or its neighbours may play a role in DM2. To unravel molecular parallels and divergences in the pathogenetic mechanisms leading to both disorders, we have analysed the expression profile of cells and tissues from DM2 patients of the ZNF9 gene and of a set of genes involved in the DM1 phenotypic outcome (CUGBP-1, Sp1, Sp2, Clcn1). We utilized in vitro cell cultures (EBV-transformed lymphocytes) from 5 DM2 patients and 3 unaffected controls. We observed a relative stability in the nuclear retention of ZNF9 mutated transcripts that does not alters the levels of ZNF9 in DM2 cells as verified by QRT-PCR and further corroborated by Western blot analysis, using a polyclonal antibody against the mammalian ZNF9 protein developed by our group. A significant misregulation was instead observed in the CUGBP1, Sp1 and to a lesser extent Sp2 genes expression in lymphoblasts from DM2 patients compared to controls. To determine if this transcriptional alteration could have an effect on the amount of the Clcn1 transcript, we performed QRT-PCR experiments on cDNA from muscle biopsy samples of 3 DM2 patients and 2 controls with a Clcn1-specific assay. Similarly to what already observed in DM1 tissues, DM2 muscle cells showed a marked reduction in Clcn1 mRNA levels of 2.5-3 fold (to 30%-40% of controls). Our results indicate that the DM1 and DM2 overlapping clinical phenotypes may derive, at least partially, from a common trans acting mechanism which traps and misregulates shared genes and proteins.

Mosaicism of a 313 bp deletion and premutation in the *FMR1* gene. P. Limprasert, T. Sripo, C. Charalsawadi, J. Wirojanonte. Faculty of Medicine, Prince of Songkla Univ, Songkhla 90110, Thailand.

The fragile X syndrome (FXS) is the most commonly inherited cause of mental retardation. An affected individual, full mutation, has an increased number of CGG repeats (> 200) and hypermethylation in the *FMR1* gene resulting in absence of the FMR1 protein (FMRP). Genetic diagnosis of this disease relies on the detection of CGG repeat in the *FMR1* gene. The length of the CGG correlates with the repeat instability. Instability may lead to mosaic conditions with a full mutation and premutation or rarely with normal or reduced alleles. We describe a 12-year-old boy who was referred for FXS testing because his younger brother had been diagnosed as a full mutation FXS. He had no striking FXS phenotype except his IQ was 75 (WISC). PCR analysis showed ~100 CGG repeats. Southern blot (EcoRI/EagI with StB12.3 probe) showed a hybridization signal at 3.1 kb corresponding to the unmethylated premutation allele and an additional band of 4.9 kb. We expected that this 4.9 Kb might be a deletion allele. Therefore, we used additional restriction enzymes (HindIII and PstI) that located around the CGG repeat region suggesting the deletion was in the proximal part of CGG repeats. The DNA when digested with EcoRI and NruI and hybridized with StB12.3 probe produced 3.2 kb (unmethylated permuation) and 2.6 kb bands in the patient suggesting the deletion site was distal to the NruI site. We designed primers to amplify the PCR product of 1283 bp in normal control while PCR product of the patients DNA was 1283 and ~970 bps. Direct sequencing analysis from the small PCR product demonstrated a 313 bp deletion and 26 CGG repeats. The breakpoint was located at position 13518 (Gene bank # L29074) and G(CG₂₆). The deletion included the Chi like-element (GGTGGAGG) and the EagI site. The most likely distal break point occurred within the expanded CGG repeat. The premutation allele was assumed to express the FMRP whereas the deletion of the promoter of *FMR1* gene was likely to cause the absence of FMRP. Because of the mosaic pattern, the patient had a mild FXS phenotype. To our knowledge, this is the first mosaicism of premutation and microdeletion in the *FMR1* gene.

FXTAS and SCA10 in American patients with movement disorders. A.I. Seixas^{1, 2}, M. Lin¹, A. Ahuja¹, C. Callahan¹, M. Maurer^{1, 3}, I. Silveira², R.L. Margolis¹. 1) Dpt. of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD, USA; 2) UnIGENe, IBMC, University of Porto, Portugal; 3) Dpt. of Physiology and Pathophysiology, University of Heidelberg, Germany.

Fragile X-associated tremor/ataxia syndrome (FXTAS) was recently described in carriers of premutation expansions (55-200 CGGs) in the Fragile X mental retardation gene 1 (*FMR1*). The syndrome is characterized by cerebellar ataxia and intention tremor. The estimated prevalence of premutations in the *FMR1* gene in the general population is 1/813 males and 1/259 females. Recent studies have suggested that these premutations may account for 5% of the cases of sporadic ataxia in adult males. Spinocerebellar ataxia type 10 (SCA10) is a late onset neurodegenerative disorder caused by massive expansions of an ATTCT repeat in the *SCA10* gene. So far, SCA10 has only been described in Mexican and Brazilian pedigrees.

We examined the frequency of FXTAS and SCA10 among patients with movement disorders of unknown cause referred to Johns Hopkins for genetic testing. Genetic analysis for the two *loci* was performed by fluorescent repeat-primed PCR assay for SCA10 (Matsuura and Ashizawa, 2002; Cagnoli *et al*, 2004), and PCR optimized for amplification of GC-rich fragments for FXTAS.

All 209 patients studied for the SCA10 mutation tested negative. Of the 166 individuals (81 males, 85 females, 127 with cerebellar disease, and 60 with basal ganglia disease) tested thus far for the *FMR1* repeat length, only one has an expansion, of approximately 120 CGGs. This premutation carrier is a 51 year old man with an intention tremor and a mild gait abnormality, and a brain MRI revealing cortical and cerebellar atrophy, with an abnormal signal in the middle cerebellar peduncle. These findings are consistent with the diagnosis of FXTAS, though his deceased father was reported to have had a tremor and seizures late in life. Our results confirm that SCA10 is a very rare disease, possibly confined to specific ethnic groups. Our results also suggest that FXTAS is a rare cause of genetically unexplained movement disorders in the American adult population.

Identification and sizing of GAA trinucleotide repeat expansion of Friedreich's Ataxia in 15 Iranian patients. *M. Shafa Shariat Panahi, M. Houshmand.* Medical Genetics Department, National Institute for Genetic engineering and Biotechnology, Tehran, Iran, P.O.Box:14155-6343.

Friedreich's ataxia is a neurodegenerative disorder whose clinical diagnostic criteria for typical cases include a) early onset age, b) autosomal recessive inheritance c) progressive ataxia of limbs and gait and d) absence of lower limb tendon reflexes. It is the commonest genetic cause of ataxia and is associated with the expansion of a GAA repeat in intron 1 of the frataxin gene. Approximately 96% of patients are homozygous for this expansion mutation with 4% being compound heterozygotes for the repeat expansion and a point mutation. We studied 15 Iranian patients (9 females and 6 males) from 7 unrelated families. DNA from each patient was extracted and frequency and length of (GAA) n repeat in the first intron of the gene FRDA was analyzed using a long-Range PCR test. Also we investigated impact of GAA size on neurological findings, age of onset and disease development. Homozygous GAA expansion was found in 12 cases (80 %) all typical cases. In 3 cases (20 %), no expansion was observed, ruling out the diagnosis of Friedreich's ataxia. In cases with GAA expansions, ataxia, scoliosis and pes cavus, cardiac abnormalities and some neurological findings occurred more frequently than in our patients without GAA expansion. Molecular analysis was imperative for diagnosis of Friedreich's ataxia, not only for typical cases, but also for atypical ones. Diagnosis bases only on clinical findings is limited, however, it aids in better screening for suspected cases that should be tested.

Search for the genetic cause of ataxia in a family without living affected patients resulted in an intermediate sized SCA2 allele. *C.C. VerschuurenBemelmans¹, E.R.P. Brunt², R.P. Stulp¹, H.H. Lemmink¹.* 1) Department of Clinical Genetics, Groningen University Hospital, P.O. Box 30001, 9700 RB Groningen The Netherlands; 2) Department of Neurology, Groningen University Hospital.

Mutations in more than 10 different genes have been identified in patients with autosomal dominant cerebellar ataxia (ADCA). Spinocerebellar ataxia type 2 (SCA2) is caused by the expansion of an unstable CAG repeat of the SCA2 gene encoding a polyglutamin tract. Wildtype alleles range from 14 to 31 CAG repeats. Mutant alleles range from 34 to more than 450 CAG repeat units. 32 and 33 CAG repeats are considered to be intermediate repeats. Wildtype CAG repeats are almost always interrupted by one or two CAA triplets whereas expanded SCA2 alleles contain nearly always uninterrupted CAG repeats units. A 52 year old woman contacted us with questions concerning ataxia in her family. Her father started to show clinical features of ataxia at an age of about 30 years. Her uncle started in his mid twenties and her aunt and grandfather around 45 years of age. All patients in this family passed away. In the third generation all eleven individuals are older than 45 years of age and have no symptoms. Since affected family members were not available anymore, only presymptomatic testing was possible for the known genes. Three persons were willing to cooperate in anonymous DNA testing. An intermediate sized SCA2 allele of 33 CAG repeats was identified in one. Sequence analysis of the expanded allele showed a 33 CAG repeat without the presence of CAA interruption. Neurologic examination showed no symptoms. In the literature 2 reports describe a young asymptomatic person, one with 32 and one with 33 repeats, who inherited this allele from their symptomatic parent with 40 repeats. A mother and daughter are reported who both carried alleles with 33 CAG repeats and developed a mild ataxia phenotype later life. Although we can not rule out that our finding is a coincidence and that another unknown gene is the cause of the ataxia in this family, it is likely to suggest that patients in this family carried more than 33 CAG repeats and that during transmission to the next generation a shorter SCA2 allele was passed on.

Autosomal dominant macular degeneration in a *Cynomolgus* monkey (*Macaca fascicularis*) pedigree: Linkage and mutation analysis. R. Ayyagari¹, S. Umeda², R. Allikmets³, A.J. Karoukis¹, R. Ambasudhan¹, H. Okamoto², M.T. Suzuki⁴, K. Terao⁴, A. Mizota⁵, Y. Yoshikowa², Y. Tanaka², T. Iwata². 1) Dept Ophthalmology, Univ Michigan, Ann Arbor, MI; 2) National Institute of Sensory Organs, National Tokyo Medical Center, Tokyo, Japan; 3) Columbia University, New York, NY; 4) Tsukuba primate center for medical science, Tsukuba, Japan; 5) Chiba university graduate school of medicine, Chiba, Japan.

Purpose: To identify the gene associated with early onset macular degeneration with drusen in the macular region segregating in a large *Cynomolgus* monkey pedigree. Histopathological analysis revealed deposits between the RPE and Bruch's membrane. **Methods:** We cloned the monkey homologues of human macular degeneration genes ABCA4, VMD2, TIMP3, EFEMP1, and ELOVL4. Mutation analysis was performed by amplification of exons using DNA from 6 affected and 11 normal monkeys followed by single strand confirmation polymorphism analysis and sequencing. Microsatellite markers linked to human macular degeneration loci were amplified using DNA from 20 affected monkeys and 6 unaffected relatives. Linkage analysis was carried out using MLINK program and affecteds-only model. **Results:** Significant homology was observed between the monkey and human homologs of the five macular degeneration genes cloned. No pathogenic changes were observed in these genes. Linkage analysis did not reveal significant positive LOD scores with markers linked to human macular degeneration loci STGD1, STGD3, STGD4, ARMD1, DHRD, Sorsby's fundus dystrophy, VMD2, MCDR1, CORD5, CORD8, and CORD9. Haplotype analysis of these candidate gene regions further supported exclusion of these loci for harboring the disease-causing gene. **Conclusions:** Involvement of the ABCA4, VMD2, TIMP3, EFEMP1, and ELOVL4 loci has been excluded in the monkey pedigree by linkage, haplotype and mutation analysis. Furthermore, by linkage analysis, we have also excluded the loci for STGD1, STGD4, ARMD1, MCDR1, CORD5, CORD8 and CORD9 conditions showing phenotypic overlap with the disease observed in affected monkeys. Macular degeneration in our monkey pedigree is likely to be due to mutations in a novel gene.

A novel missense mutation in the OPA1 gene found in a large Iranian family with Autosomal Dominant Optic Atrophy (ADOA). *s. dadgar*^{1,2}, *m. garshasbi*², *e.n. haghghi*², *s. schimpf*³, *b. wissinger*³, *s. dadgar*⁴, *o.e. khorasani*⁵, *y. shafeghati*², *h. najmabadi*². 1) Best Medical Research , Charles H. Best Institute, University of Toronto, Ontario, Canada; 2) Genetics Research Center, The Social Welfare and Rehabilitation Sciences University, Tehran, Iran; 3) Molecular Genetics Laboratory at the University Eye Hospital, Tbingen, Germany; 4) George Washington University Medical Center, Washington DC, USA; 5) Clinical Skill Lab Center, Medical Science University, Firoozgar Hospital, Tehran, Iran.

Autosomal dominant optic atrophy (ADOA) is the most frequent form of hereditary optic neuropathies with an estimated disease prevalence ranging between 1:12000 and 1:50000 in different populations. We describe here a large Iranian family with 40 affected individuals (31 alive and 9 dead) showing a dominant way of inheritance of optic atrophy. For none of the patients pre-, peri- or postnatal problems have been reported. On average, at the age of 14 the visual acuity of the patients began to decrease and progressed gradually. In almost all of the patients the vision is about 10/100. Some of them have been tested by perimetry revealing central scotoma. The study of visual evoked potentials (VEPs) showed prolonged latency and decreased amplitude. These and the diagnostic findings from fundoscopy and retinal angiography support the diagnosis of optic atrophy. A major locus for optic atrophy maps to chromosome 3q28 containing the gene OPA1. Using five microsatellite markers from chromosome 3 (D3S2398, D3S2418, D3S3562, D3S1265 and D3S1311) we could confirm their cosegregation with the disease. By direct sequencing of exons 8 to 16 and 27 from OPA1 we have identified a novel heterozygous missense mutation (c.1313A>G) at the first position of exon 14 replacing the normal Aspartic acid by a Glycine (Asp438Gly). At the same position another missense mutation (1313A>T; Asp438Val) has been reported before, indicating the importance of this position in OPA1.

DENATURING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (DHPLC) FOR THE MUTATIONAL ANALYSIS OF THE SPAST GENE. A. Magariello¹, R. Mazzei¹, F.L. Conforti¹, A. Patitucci¹, A.L. Gabriele¹, G. Peluso¹, C. Ungaro¹, T. Sprovieri¹, M. Mancuso², S. Di Mauro², D. Branca³, U. Aguglia³, M. Muglia¹. 1) Inst Neurological Sci, National Research Council, Mangone, Cosenza, Italy; 2) Dept of Neurology, Columbia University College of Physicians and Surgeons, New York, USA; 3) Regional Epilepsy Center, A.O. Bianchi Melacrino Morelli, Reggio Calabria, Italy.

The hereditary spastic paraplegia (HSP) is characterized by progressive spasticity and hyperreflexia of the lower limbs. Clinically HSP is classified as pure or complicated form. Autosomal Dominant (AD), Autosomal Recessive (AR) and X-linked inheritance occur in both forms. Pure ADHSP is probably the most common form (40%) and up to date 10 loci have been identified. This form is caused by mutations in the SPAST gene that is located on chromosome 2p (SPG4 locus), comprising 17 exons and encodes the protein spastin, a member of the AAA family of adenosine triphosphatases. Over 100 spastin mutations include missense, nonsense and splice site point mutations, as well as little insertions and deletions. To date, the SPAST gene mutations have been revealed by SSCP analysis or by direct sequencing of cDNA. In this study we described a new method for the rapid screening of the SPAST gene by DHPLC. We performed screening mutations by DHPLC in three families with SPG4-linked HSP. In two families heteroduplex DHPLC profiles were observed in exon 5 and exon 15 respectively. Direct sequencing revealed two previously described stop codon mutations (R562Stop and T269Stop). In the last family different heteroduplex DHPLC profiles were observed in the exons 6 and 14. Direct sequencing of the exon 6 showed an already described G>A transition (P879P) and the sequence analysis of exon 14 revealed a new polymorphism in intron 13 (IVS1310). Because no additional heteroduplex patterns were observed in this family, we performed a direct sequence of the spastin, but no variations have been detected, confirming the DHPLC results. The use of DHPLC for mutation detection represents a significant advantage in the molecular diagnosis of HSP caused by mutation in the SPAST gene and it should be used for the rapid screening of a large number of patients.

Identification of genes underlying hypodontia. *P.I. Patel*¹, *P. Tarpey*², *G. Mendoza*¹, *C. Gonzales*¹, *P. Das*³, *V. Ninis*³, *D.W. Stockton*⁴, *R. Scarel-Caminaga*⁵, *L. Nino-Rosales*³, *E. Figuerido*⁶, *S. Leal*⁴, *M.R. Stratton*², *S. Line*⁵. 1) Institute for Genetic Medicine, University of Southern California, Los Angeles, CA; 2) The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, UK; 3) Department of Neurology, Baylor College of Medicine, Houston, TX; 4) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 5) Faculty of Odontology of Piracicaba-UNICAMP, Brazil; 6) Federal University of Alagoas, Maceio, Brazil.

The congenital absence of teeth is a common developmental anomaly of human dentition that affects approximately 20% of the population. Although new genetic and molecular approaches have increased our understanding of the molecules that control tooth patterning (number, position, shape and size), the precise nature of the genes underlying congenitally missing teeth or hypodontia remains poorly understood. Hence, understanding the molecular basis for missing teeth is an important goal, that is both timely and significant to the practice of orthodontics. Our long-term goal is to elucidate factors important for tooth development in humans. We have sampled large families segregating autosomal dominant hypodontia involving a single premolar or premolars and canines. Total genome scans have been completed and fine-mapping is in progress for these families, as well as mutation analysis in candidate genes. We have also mapped the locus for an X-linked dominant form of hypodontia involving incisors to Xp11.22-Xq13.1. High-throughput sequencing is being used to identify the gene underlying this condition and these results will be presented. (Supported by NIH grant DE14102, Wellcome Trust and Center for Inherited Diseases Research).

The positional candidate genes ENO1 and RBP7 do not harbor mutations in the coding sequence for Schnyder's crystalline corneal dystrophy. *V.P. Theendakara¹, G. Tromp¹, H. Kuivaniemi^{1, 2}, P.S. White^{5, 6}, R.S. Winters⁵, J.S. Weiss^{3, 4}.* 1) CMMG, Wayne State University School of Medicine, Detroit, MI; 2) Department of Surgery, Wayne State University School of Medicine, Detroit, MI; 3) Department of Ophthalmology, Wayne State University School of Medicine, Detroit, MI; 4) Kresge Eye Institute, Wayne State University School of Medicine, Detroit, MI; 5) Division of Oncology, Children's Hospital of Philadelphia, University of Pennsylvania, PA; 6) Department of Pediatrics, University of Pennsylvania, PA.

Schnyder's crystalline corneal dystrophy (SCCD) is a rare autosomal dominant eye disease. The clinical manifestations of the disease may include bilateral corneal clouding, arcus lipoides and anterior corneal crystalline cholesterol deposition. A genome wide scan with two large Swede-Finn families mapped the SCCD locus to 1p36 (Shearman et al. *Hum Mol Genet* 5: 1667- 1672, 1996). Eleven additional families were included to narrow the critical region for SCCD. Recombination-based haplotype analysis refined the candidate interval to 2.32 Mb between markers D1S1160 and D1S1635 (Theendakara et al. *Hum Genet* 114: 594-600, 2004). Within this region there are twenty known and nine putative genes. Biologically plausible candidate genes that are expressed in the eye such as ENO1 (enolase1, LocusID 2203), encoding both a glycolytic enzyme and a lens structural protein, and RBP7 (retinol binding protein, LocusID 116362), which belongs to CRBP family that interacts with small binding proteins, were analyzed. The first phase of sequencing was performed in the coding regions and the exon-intron boundaries, to detect sequence variations and splice site mutations. Three sequence variants (IVS1G+ 61C, IVS2T+51G and IVS5C-9T) were detected in ENO1 gene. Affected individuals did not have any sequence changes in the coding regions, with respect to the reference genomic sequence (NT_021937) in either ENO1 or RBP7. These findings suggest that neither ENO1 nor RBP7 are involved in SCCD.

Evaluating a potential role for the POLK gene product in female infertility and/or ovarian failure. *L.D. McDaniel, R.A. Schultz, E.C. Friedberg.* Department of Pathology, Univ Texas SW Medical Ctr, Dallas, TX.

Female infertility and ovarian failure are significant medical concerns within the human population and represent subjects in search of candidate genes. DNA polymerase kappa (POLK), a member of the recently discovered Y family of DNA polymerases, that in vitro have demonstrated translesion synthesis past DNA damage that has stalled the normal replicative polymerases. This process has been shown to be relatively error prone. Bypass polymerases, but not POLK, have been shown to participate in the introduction of nucleotide changes in the immunoglobulin genes during the process somatic hypermutation. POLK protein has also been shown to bypass thymine glycol, Acetylaminofluorene, and benzo[a]pyrene adducts but not UV induced adducts in DNA. The human POLK gene is expressed at high levels in the seminiferous tubules of the testis, the ovaries and adrenal cortex, as well as at lower levels in most other tissues. The expression pattern of POLK makes it an interesting candidate for specific roles in germinal tissues. We have a knockout mouse model for POLK to evaluate the phenotypes. Mutant animals are viable and exhibit no overt phenotypes. Given the expression pattern, we examined the fertility of Polk mice. Crosses between Polk mutant or heterozygous males and wild type females yield an average of 8 pups per litter, equivalent to that seen in wild type crosses. In contrast, crosses between Polk females and wild type, heterozygous, or mutant males yielded equivalent results with litters of 3 to 4 pups per litter. Heterozygous females crossed to any genotype produce an intermediate litter size. Lost animals represent all genotypes equally. Thus, deficiency in Polk protein, including haploinsufficiency, yields a female-specific reduction in fertility, due to a compromise in the ability of females to initiate and/or develop fetuses to term. Quantitation of implantation rate and age of fetal demise will be presented. The possible function(s) for Polk in the adrenal cortex versus the ovary will be discussed. Additionally, approaches to role(s) for POLK mutations or polymorphisms in human infertility and/or ovarian failure and fetal demise will be discussed.

Cirhin, the protein mutant in NAIC is a transcriptional cofactor of HIVEP1 DNA binding protein. *B. Yu, G.A. Mitchell, A. Richter.* Division of Medical Genetics, Hôpital Sainte-Justine, Montréal, QC, Canada.

North American Indian childhood cirrhosis (NAIC/CIRH1A, OMIM 604901) is a severe, autosomal recessive intrahepatic cholestasis mapping to 16q22. All NAIC patients tested to date are homozygous for a mutation that changes a conserved arginine at position 565 to tryptophan (R565W) in cirhin, the 686 amino acid protein product of the locus. We determined that cirhin is a nucleolar protein. It has an active C-terminal monopartite nuclear localization signal (NLS) and a unique nucleolar localization signal. While R565W alters the predicted secondary structure of cirhin, it has no effect on subcellular localization (Yu et al, in preparation). Yeast 2 hybrid screening against human liver cDNA library revealed a single interaction partner (CIRIP, cirhin interaction protein). It is the 684 aa C-terminal fragment of HIVEP1 containing the 2nd C2H2 zinc finger domain and a domain rich in acidic residues. The R565W mutation decreased the interaction. HIVEP1 is a C2H2 zinc finger DNA binding protein that regulates transcription by binding to similar NF- κ B motifs such as those found in HIV-1 LTR, p53 and IRF-1. To validate the observed interaction between cirhin and CIRIP, they were subcloned into pcDNA3.1- and the HIV-1 LTR enhancer sequence (GGGACTTTCC) into the PGL3 promoter vector. Our results show that individually cirhin and CIRIP can significantly increase the transcriptional activity of luciferase (CIRIP: 40-70%, cirhin: 20-50%, compared to control) in HeLa cells. Thus, in contrast to claims that only full length HIVEP1 has function on the HIV-1 LTR enhancer, we find that CIRIP is also active. Co-transfection of Cirhin and CIRIP increases luciferase activity more significantly (80-150 %). Our current hypothesis is that cirhin binds to endogenous HIVEP1 or other interaction partners, and increases the transcriptional activity of the HIV1 LTR enhancer in HeLa cells. The role of R565W on this transcriptional enhancer is now under investigation. The effect of CIRIP on HIV1 enhancer supports the idea that it may be a functional alternative splice product of HIVEP1. Supported by the Canadian Institutes of Health Research.

Loss of the Shwachman-Diamond syndrome gene (*SBDS*) causes early embryonic lethality. *S. Zhang*^{1, 2}, *M. Shi*², *J.M. Rommens*^{1,2}. 1) Dept. of Molecular and Medical Genetics, University of Toronto, Toronto, ON, Canada; 2) Program in Genetics & Genomic Biology, The Hospital for Sick Children, Toronto, ON, Canada.

Shwachman-Diamond syndrome (SDS, MIM 260400) is a multi-system autosomal recessive disorder caused by mutations in the *SBDS* gene. Individuals with SDS display clinical features of exocrine pancreatic insufficiency, hematological dysfunction and skeletal abnormalities. *SBDS* is a highly conserved gene of unknown function with homologs in vertebrates, plants and archaea. Indirect evidence implicates a role in RNA metabolism. In the mouse, *SBDS* expression is broad and variable with highest levels in the early embryo. To elucidate the function of *SBDS*, we have developed an animal model using gene targeting techniques. A null allele was generated by insertion of an *IRES-lacZ-PGK-Neo* cassette in exon 1 to lead to interruption of translation. Mice, with one interrupted allele (*SBDS*^{-/+}), are phenotypically indistinguishable from their homozygous wild type littermates (*SBDS*^{+/+}) in viability, growth and fertility. No homozygous mutant mice (*SBDS*^{-/-}) were born of more than 20 *SBDS*^{-/+} intercross matings while 74 mice (65%) were heterozygous and 39 mice (35%) were homozygous for the wild type allele. Abnormal embryos were found at developmental stage 8.0-8.5dpc with the *SBDS*^{-/-} genotype, indicating that loss of *SBDS* leads to early embryonic lethality. These findings are consistent with the absence of homozygosity of common early truncation alleles in SDS patients and suggest a critical role for *SBDS* in early development.

Cellular, spatial, and temporal expression of developmentally regulated GTP-binding protein 2 (*DRG2*). V.A. Vinoverski¹, C.N. Vlangos², S.H. Elsea^{1,2,3}. 1) Dept. of Zoology, Michigan State University, East Lansing, MI; 2) Genetics Graduate Program, Michigan State University, East Lansing, MI; 3) Depts. of Human Genetics and Pediatrics, Virginia Commonwealth University, Richmond, VA.

Smith-Magenis syndrome (SMS) is a multiple congenital anomalies and mental retardation disorder with a distinct phenotype characterized by craniofacial, skeletal, neurological, and sleep abnormalities, in addition to maladaptive behaviors including self-injury and explosive temper. SMS is usually associated with an interstitial deletion of chromosome 17p11.2. We previously characterized SMS patients without a FISH detectable 17p11.2 deletion who carry dominant frame-shift mutations in the *RAI1* gene, which maps to chromosome 17p11.2. While it is likely that haploinsufficiency of *RAI1* is responsible for most of the SMS phenotype, SMS patients with *RAI1* mutations have not been found to have many of the more variable features often seen in patients with a 17p11.2 deletion. Thus, additional genes mapping to 17p11.2 may contribute to these less penetrant characteristics of SMS. *DRG2*, a member of the GTPase superfamily, maps to the SMS critical region. The mouse ortholog of human *DRG2* maps to chromosome 11 in the region syntenic to human chromosome 17p11.2. In order to understand the biochemical role of *DRG2/Drg2*, we examined the cellular localization of *Drg2* in COS-7 cells through use of a GFP-fusion construct. GFP-*Drg2* fusion protein was detected in the golgi apparatus and endoplasmic reticulum. GFP-*Drg2* was also present in small protein bundles throughout the cytoplasm and at the junctions between neighboring cells. We also studied the spatial and temporal expression of *Drg2* during development by immunohistochemistry (IHC) on sectioned mouse embryos from embryonic day eight through birth. Throughout mouse development, the IHC studies showed high levels of *Drg2* expression in the developing brain and spinal cord. Staining was also seen in numerous spinal ganglia. These data suggest a possible role in cellular communication and/or molecular trafficking between the golgi apparatus and the cell membrane especially in the developing neural tissue.

Deletions of conserved non-genic sequences (CNGs) upstream and downstream of *FOXL2* as a novel disease-causing mechanism in BPES. *D. Beysen*¹, *B.P. Leroy*¹, *A. Lucassen*², *J. Yates*³, *J. Clayton-Smith*⁴, *H. Ilyina*⁵, *S. Sklower Brooks*⁶, *S. De Jaegere*¹, *L.M. Messiaen*⁷, *A. De Paepe*¹, *E. De Baere*¹. 1) Ctr Med Genet, Ghent Univ Hosp, Ghent, Belgium; 2) Wessex Clin Genet Service, PAH, Southampton, UK; 3) Dept of Med Genet, Addenbrooke's Hosp, Cambridge, UK; 4) St Mary's Hosp, Manchester, UK; 5) Beloruss Inst for Hered Dis, Minsk, Russia; 6) NY State Inst for Basic Research, NY, USA; 7) Dept of Genet, UAB, Birmingham, AL, USA.

The expression of a gene requires not only the presence of an intact coding sequence, but also a correctly functioning regulatory control. Regulatory elements can be located at large distances from transcription units on which they exert their effect, as shown in several developmental genes. In previous studies of the developmental disorder blepharophimosis syndrome (BPES, MIM 110100), an eyelid malformation associated or not with premature ovarian failure (POF), we identified a molecular defect in 70% of BPES patients (76 intragenic *FOXL2* mutations and 8 (micro)deletions including *FOXL2*). Here, we show that BPES results from deletions located outside the transcription unit of *FOXL2*. Of the 5 deletions identified 4 were located at a minimal distance of 100 kb upstream of *FOXL2*, being telomeric to 3 known translocation breakpoints in BPES patients. These deletions were found in two sporadic BPES cases and in two BPES type II families with normal psychomotor development. Their shortest region of overlap (SRO) is 100 kb and contains several highly conserved non-genic sequences (CNGs) present in human, mouse, chicken and goat. Intriguingly, the human orthologous region of an 11.7 kb goat sequence deleted in the PIS goat, considered as an animal model for BPES, is also contained in this SRO. In addition, one 150-kb deletion was found at 13 kb downstream of *FOXL2* in a BPES family where germinal mosaicism was proven at the molecular level in the sperm of an unaffected transmitting patient, indicating this is an important counselling issue. In conclusion, we postulate that *FOXL2* transcription is altered by removal of these CNGs, acting as long-range *cis* regulatory elements of gene expression and we propose this as a novel mechanism underlying BPES.

Four Common Glomulin Mutations Cause Two-Thirds of Glomuvenous Malformations (Familial Glomangiomas): Evidence for a Founder Effect. P. Brouillard¹, M. Ghassib¹, A. Penington², L.M. Boon¹, A. DompMartin³, K. Temple⁴, M. Cordisco⁵, D. Adams⁶, F. Piette³, J. Harper⁴, S. Syed⁴, F. Boralevi³, A. Tab³, S. Danda², E. Baselga⁷, O. Enjolras⁸, J.B. Mulliken⁹, M. Vikkula¹. 1) Christian de Duve Inst & UCL, Brussels, Belgium; 2) Univ of Melbourne, St. Vincent's Hospital, Australia; 3) CHU Caen & CHU Bordeaux & CHRU Lille, France; 4) Southampton Univ & Hospital NHS Trust & Great Ormond Street Hospital for Children NHS Trust, UK; 5) Hospital Nacional de Pediatria, Argentina; 6) Children's Hospital Medical Center, Cincinnati, USA; 7) Hospital de la Santa Creu i Sant Pau, Spain; 8) Hôpital Lariboisière, France; 9) Children's Hospital, Boston, USA.

Glomuvenous malformation (GVM) is a localized cutaneous vascular lesion histologically characterised by abnormal smooth muscle-like glomus cells in the walls of distended endothelial-lined channels. We linked inheritable GVMs to chromosome 1p21-22 (Boon *et al.* 1999), and found the cause to be truncating mutations in *glomulin* (*glmn*) (Brouillard *et al.* 2002). *Glomulin* expression is restricted mainly to vascular smooth muscle cells during murine development (McIntyre *et al.* 2004). In addition, we identified a double-hit mutation in one lesion (Brouillard *et al.* 2002). This finding supports our hypothesis that GVM is due to complete localized loss of function and explains the paradominant mode of inheritance. Here, we report on the identification of a mutation in *glomulin* in 23 additional families with GVM. Three mutations are new; the others have been described previously. Among the 17 different inherited mutations in *glomulin* known to date in 43 families, the 157delAAGAA mutation is the most frequent and was present in 21 families (48.8%). One mutation was found in 5 of the families (11.8%) and two other ones were determined in two families (4.7% each). Polymorphic markers suggested a founder effect for all four mutations. Screening for these mutations should lead to a genetic diagnosis in about 70% of patients with inherited GVMs. So far, a mutation in *glomulin* has been found in all GVM families tested, thus demonstrating locus homogeneity. (vikkula@bchm.ucl.ac.be) (<http://www.icp.ucl.ac.be/vikkula>).

Haplotype structure and linkage disequilibrium across the X-linked Chronic Granulomatous Disease gene (CYBB) in human populations. *E.M. Tarazona-Santos, T. Bernig, S.J. Chanock.* Section of Genomic Variation, Pediatric Oncology Branch, National Cancer Institute/NIH, Gaithersburg, MD.

Chronic granulomatous disease (CGD) is a hereditary, primary immunodeficiency characterized by an increased susceptibility to bacteria and fungi infections due to a defect in the phagocytic respiratory burst. CGD is caused by a mutation in one of four genes in the phagocyte NADPH oxidase. Nearly seventy percent of CGD cases are X-linked and due to mutations in the CYBB gene (mapped to Xp21.1), which codes for the trans-membrane gp91 component of the phagocyte NADPH oxidase. The CYBB gene is 33kb in size and contains 13 exons. We sequenced 14 kb of the CYBB gene (including 2 kb of the 5flanking region and all 13 exons) in 102 healthy individuals from four self-described ethnic groups of the SNP500cancer program (<http://snp500cancer.nci.nih.gov>); this includes 31 European, 24 African, 23 Hispanics and 24 Pacific Rim subjects. We validated 14 SNPs in CYBB with bi-directional sequence analysis; of these, there is only one coding variant, a previously described non-synonymous C/T transition at position 23817 in exon 9. We determined the haplotype structure as well as the pattern of linkage disequilibrium in the four ethnic groups using common SNPs (i.e., frequency greater than 10%); overall, there is a substantial difference in haplotype frequencies in CYBB. Six SNPs were shared by more than one population, whereas 8 are population-specific (six in subjects of African ancestry). Further haplotype analysis demonstrates different patterns of genetic diversity and linkage disequilibrium between African and non-African groups. Knowledge of the haplotype structure should be useful to investigate the possibility that rare-family mutations in CYBB (resulting in CGD) could preferentially arise on select background haplotypes. Moreover, because the phagocyte NADPH oxidase is an integral component in host defenses against pathogens, haplotype-tagging SNPs in CYBB gene should be applied to candidate gene association studies, especially targeting infectious and auto-immune diseases.

Delineation of Cohen Syndrome following a large-scale genotype-phenotype screen. *K.E. Chandler¹, R. Wilkinson², F.D.C. Manson^{1,2,3}, J. Kolehmainen^{4,5}, J. Clayton-Smith¹, A-E. Lehesjoki^{4,5,6}, G.C.M. Black^{1,2,3}.* 1) Academic Unit of Medical Genetics and Regional Genetics Service, University of Manchester, Manchester, UK; 2) Academic Unit of Ophthalmology, University of Manchester, Manchester, UK; 3) Centre for Molecular Medicine, University of Manchester, Manchester, UK; 4) Folkhäsen Institute of Genetics, University of Helsinki, Helsinki, Finland; 5) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 6) Neuroscience Centre, University of Helsinki, Helsinki, Finland.

Cohen syndrome (CS) is a rare autosomal recessive condition characterised by developmental delay, facial dysmorphism, retinal dystrophy and neutropenia. This pleiotropic phenotype coupled with a lack of full clinical data can lead to misdiagnosis, creating confusion in the literature. We have proposed precise diagnostic criteria following detailed clinical studies in the UK and Finland and found that COH1, a novel widely expressed gene, is mutated in a number of these patients.

We have now performed a comprehensive genotype-phenotype study in 59 patients with a provisional diagnosis of CS, the largest cohort of CS patients assembled to date. We found 22 different COH1 mutations in 28 of 37 probands identified as having CS by our diagnostic criteria. An additional 3 novel mutations were identified in probable CS patients with incomplete clinical data. The majority of mutations are predicted to result in a null allele through frameshift or nonsense sequence alterations. In the 22 patients with a provisional diagnosis of CS, but who did not fulfil the diagnostic criteria, only one patient had a heterozygous sequence alteration, which was not identified in 192 control chromosomes.

This study demonstrates that predicted pathogenic sequence alterations are only associated with CS patients and that there is no apparent genotype-phenotype correlation within this group. It validates our proposed diagnostic criteria for the accurate diagnosis of CS and provides a basis for the laboratory screening of COH1.

Genomic Rearrangements in the CFTR gene: results of a worldwide survey; analysis of mutational mechanisms.

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Cystic Fibrosis is caused by mutations in the Cystic Fibrosis conductance Regulator gene (CFTR/ABCC7). Over 1300 mutations have been so far identified, most of these being point mutations. We have recently reported (Audrezet et al. Human Mutation 2004) that gross genomic rearrangements could account for a part of unidentified alleles. In the present work we have set up a large collaborative study including CF chromosomes with at least one unidentified allele after an exhaustive scanning of the coding sequence of the gene (CF chromosomes were from Spain, Ireland, Scotland, Czech Republic, Italy, Belgium, France, Australia, America United States) using a QMPSF technique (Quantitative Multiplex PCR of short fragments). With the exception of Tunisia (absence of deletion), in these different populations, we have found between 10 to 20 % of deletions/insertions depending on the origin of the CF chromosomes. The breakpoint of the deletions were precisely determined and the underlying mechanisms of mutagenesis was carefully analyzed. Complex genomic rearrangements are far more frequent than previously expected and this allows us now to propose that the ideal strategy for exhaustive detection of mutations in the CFTR gene would included a screening for the 30 common mutations, a scanning of the 27 exons by D-HPLC and a finaly scanning of the coding sequence of the gene by QMPSF. This stepwise approach should maximize the mutant allele detection rate in most populations of caucasian origin.

Involvement of EIF2B genes in a large cohort of POF patients. A. FOGLI¹, F. GAUTHIER-BARICHARD¹, R. SCHIFFMANN², V. VANDERHOOF³, V. BAKALOV³, L. NELSON³, O. BOESPFLUG-TANGUY³. 1) Facult de Mdecine, INSERM U384, Clermont-Fd, France; 2) Developmental and metabolic neurology branch, NIH, Bethesda, Maryland, USA; 3) National institute of child health and human development, NIH, Bethesda, Maryland, USA.

Premature Ovarian Failure (POF) can be expressed as a primary or secondary amenorrhea lasting more than six months, associated with elevated gonadotrophins before the age 40 years. Premature OF affects 1 percent of all women and occurs in 0.1 percent before the age of 30 years. Other than karyotype abnormalities, including various X chromosome such as Turner syndrome, very few genes are known to be associated with this ovarian dysfunction. Recently, three of the five EIF2B genes (EIF2B2, 4 and 5, eight mutations) were involved in seven patients who presented with POF and white matter abnormalities on MRI (ovarioleukodystrophy). These genes encode the five subunits of the eucaryotic initiation factor 2B (eIF2B alpha to epsilon), which is involved in the first step of the protein synthesis. In ovarioleukodystrophy, a phase without neurological symptoms and an apparently isolated form of POF could be observed. Thus, to test further the involvement of EIF2B genes in classical POF, we screened a series of 93 patients with OF but without identified leukodystrophy or neurological symptoms for these eight mutations and two additional mutations found in patients with milder forms of eIF2B-related disorders. No abnormalities were identified. We concluded that eIF2B mutations already described as causing POF associated with white matter abnormalities are an uncommon cause of spontaneous premature ovarian failure (95 percent confidence limit under 3.2 percent of cases).

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GJB2 mutations - passage through Iran. *K. Kahrizi¹, C. Nishimura³, Y. Riazalhosseini¹, M. Malekpour¹, A. Daneshi², M. Farhadi², M. Mohseni¹, N. Mahdieh¹, N. Bazazzadegan¹, A. Naghavi¹, M. Avenarius³, K. Javan¹, R.J.H. Smith³, H. Najmabadi¹.* 1) Genetics research center, Welfare & Rehab Univ, Tehran, Iran; 2) Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran; 3) Molecular Otolaryngology Research Laboratories, Department of Otolaryngology, University of Iowa, Iowa City, IA, USA.

Hereditary hearing loss (HHL) is a very common disorder. When inherited in an autosomal recessive manner, it typically presents as an isolated finding. Interestingly and unexpectedly, in spite of extreme heterogeneity, mutations in one gene, GJB2, are the most common cause of congenital severe-to-profound deafness in many different populations. In this study of the Iranian population, we assessed the contributions made by GJB2 mutations and g.1777179_2085947del (hereafter called (GJB6-D13S1830)) to the autosomal recessive non-syndromic deafness genetic load. Probands from 664 different nuclear families were investigated. GJB2-related deafness was found in 111 families (16.7%). The carrier frequency of the 35delG mutation showed a geographic variation that is supported by studies in neighboring populations. (GJB6-D13S1830) was not found. Our prevalence data for GJB2-related deafness reveal a geographic pattern that mirrors the south-to-north European gradient and supports a founder effect in south-eastern Europe.

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F508 in association with 7T - the exception that proves the rule? *D. Kennedy, T. Ashfield, J. Guo, D. Ramadeen, R. Rezaie, M. Shama, M. Skuterud, D.J. Allingham-Hawkins, H.M. Bedford.* Genetics Program, North York General Hospital, Toronto, ON, Canada.

A cord blood sample was referred for CF testing on a newborn male at 25% risk for CF. His 3.5 year old sister had been diagnosed only two weeks prior to his birth and her genotype was reported as F508/F508 by another lab. Both the baby's sample and samples from each parent were tested using the Innogenetics CFTR36 Probe Array kit which tests for 36 mutations and the 5T/7T/9T polymorphisms in intron 8. The baby was found to be neither affected nor a carrier but an unusual result was obtained with the father's sample. The father's genotype was F508/+, 7T/7T. The F508 mutation is hypothesized to have arisen in Europe 10,000 years ago (Casals *et al.*, 1992) and linkage disequilibrium has been shown with a number of polymorphisms including the 9T allele. The finding of F508 in *cis* with 7T was unusual enough to prompt a literature search, with no success, to find a similar case. The clinical significance of this finding is unknown; however, it is interesting to note that the affected child has had a relatively mild course to date. She weighed 8 lbs at birth with no meconium ileus or failure to thrive. At the time of diagnosis (approx. 3.5 years), her only overt symptom was frequent, large, oily stools. She was above the 75th percentile for weight and at the 75th percentile for height. She had not experienced more than an average number of respiratory infections nor treatment with antibiotics than expected for a child her age. There was no history of asthma. Laboratory analyses did reveal pancreatic insufficiency and vitamin E deficiency, both of which were previously undetected. Is it possible that this relatively mild clinical course is related to her genotype, specifically to having F508 in *cis* with 7T - which has a lower efficiency of appropriate exon 9 splicing than 9T - on one allele? Perhaps, but it is difficult to imagine how 7T could moderate the effect of F508 on a molecular level and this can not be determined based on a single case. Knowledge of the existence of such cases is important in the laboratory, however, especially from a quality assurance perspective.

Genotype/Phenotype correlation of 5q13 region genes in Spinal Muscular Atrophy patients from India. A.

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Spinal muscular atrophy is an autosomal recessive neuromuscular disorder, which leads to symmetrical muscle weakness and atrophy. SMA has been classified into four groups based on the age of onset and clinical severity of the disease. Homozygous deletion in SMN1 gene causes the disease but the clinical severity may be modified by copy number of homologous gene SMN2 as well as other genes in the flanking region. In the view of paucity of data regarding genotype/phenotype correlation, this study has been undertaken to determine the correlation in SMA patients by using the SMN and NAIP genes and two polymorphic markers. Clinically and neurophysiologically diagnosed SMA patients were included in the study. Gene deletion study was carried out in 49 SMA patients, 15 Segmental SMA (Hirayama) patients and 50 controls of same ethnic group. Both SMN1 and NAIP genes were homozygously deleted in 69 % and 31% respectively in classical SMA patients. No point mutations were found in exon 3 and intron 6 through exon 8 by sequence analysis of non deletional patients. Two to four alleles of the markers C212 and C272 were observed in normal individuals. However, majority of Type I patients showed only one allele from both markers whereas in Type II and III patients, 2-3 alleles were observed. The SMN2 copy number in our type III patients showed that patients carry 3-5 copies of SMN2 gene. Our results suggest that NAIP and H4F5 along with SMN2 copies may modify the SMA phenotype.

Novel and Recurrent Mutations in the NF1 Gene in Korean Patients with Neurofibromatosis Type 1. *C.H. Lee¹, H.W. Lee¹, E.S. Park¹, M.J. Lee¹, J.S. Lee^{1, 2}.* 1) Brain Korea 21 Project for Medical Science, Yonsei University, Seoul, Korea; 2) Dept Clinical Genetics, Yonsei Univ Col Medicine, Seoul, Korea.

Neurofibromatosis type 1 (NF1) is one of the most common autosomal dominant disorder with an incidence of 1 : 3,500 which is caused by mutations in the NF1 gene. NF1 is characterized particularly by cafe-au-lait spots and fibromatous tumors of the skin. The NF1 gene is located on the chromosome 17q11.2 and spans approximately 350 kb of genomic DNA and it consist of 60 exons and translates into neurofibromin. The neurofibromin comprises 2818 amino acids and has an estimated molecular weight of 327 kDa. The central region of neurofibromin (encoded by exons 21-27) possesses marked homology to Ras-GTPase activation proteins (GAPs). Screening of NF1 gene is complicated because of the large size of the gene, the presence of pseudogenes, the great variety of possible lesions, and the lack of significant mutational clustering. We screened for mutations in 26 patients who are clinically diagnosed as neurofibromatosis. The whole coding sequences and all splice sites were examined for mutations using DHPLC followed by direct sequencing of PCR products. Disruptive mutations were identified in 21 individuals with an overall mutation detection rate of 81%. The mutations included one indel, two insertions (nt. 4159, 1233), six deletions (nt., 953, 1017, 1541, 2679, 2816, 3525), thirteen missense / nonsense mutations (2237, 616, 2483, 1403, 2197, 2496, 2157, 2426, 2429, 1466, 192, 384, 386 codon) and one splicing error (IVS 25). Eight unclassified polymorphisms were also detected. Sixteen (69.5%) of the identified disruptive mutations are novel. Seven mutations have been previously reported.

Analysis of Duplication/Deletion of the PMP22 gene in patient with CMT1A and HNPP by DHPLC. *H.W. Lee¹, C.H. Lee¹, J.S. Lee^{1,2}*. 1) BK21 Project for Med. Science, Yonsei Univ., Seoul, Korea; 2) Dept Clinical Genetics, Yonsei Univ. Col Medicine, Seoul, Korea.

Charcot-Marie-Tooth disease (CMT) and hereditary neuropathy with liability to pressure palsies (HNPP) are the most frequent inherited disorders of the peripheral nervous system. They are clinically and genetically heterogeneous. A submicroscopic tandem duplication of 1.5 Mb in chromosome 17p11.2-12 comprising the PMP22 gene is found in 70.7% of autosomal dominant Charcot-Marie-Tooth type 1 (CMT1) patients. A reciprocal deletion is found in 87.6% of HNPP patients. For molecular diagnosis of duplication or deletion of the PMP22 gene, several approaches are available; Southern blot analysis, fluorescence in situ hybridization (FISH), real-time quantitative PCR analysis. However, there are several limitations in each method. To develop method for detection of 1.5 Mb duplication & deletion in CMT1A & HNPP patient we analyzed unrelated sixteen families diagnosed clinically with CMT1A, nine families with HNPP and thirty normal controls. Gene dosage of the region was determined by PCR with D17S122, D17S162, D17S261, followed by DHPLC analysis. Quantitative analysis by DHPLC showed that samples with duplication/deletion of PMP22 gene have different patterns. Absorbance (mV) was increased in CMT1A patient and decreased in HNPP patient. DHPLC analysis results consisted with Real-time quantitative PCR analysis and it showed correct determination of the PMP22 gene copy number. This method is fast, easy and reproducible in detecting PMP22 duplication and deletion in CMT1A and HNPP patients, respectively. This method might be very helpful for the diagnosis of patients with CMT1A/HNPP.

A synonymous *TCOF1* change in a patient with Treacher Collins syndrome causes missplicing of a constitutive exon due to disruption of a putative splicing enhancer. *D. Macaya, S. Katsanis, T. Hefferon, S. Audlin, G. Cutting.* DNA Diagnostic Lab, Inst of Genetic Med, Johns Hopkins Univ, Baltimore, MD.

Interpretation of novel sequence alterations in a disease associated gene remains a challenge in the clinical setting, especially for synonymous alterations of unknown consequence. We describe here the application of RNA transcript analysis to investigate the effect of a *de novo* synonymous transversion (S1127S or 3381A>C) 12bp downstream from the 3' splice acceptor of exon 22 of *TCOF1* in a patient with Treacher Collins syndrome (TCS). Inspection of the region surrounding this exonic mutation revealed a sequence with a higher consensus score for a 3' splice site than the reported site, leading to speculation of an alternate 3' splice site for exon 22. Because the 3381A>C mutation is in a critical position, it would disrupt splicing at this alternate site. To evaluate exon 22 splicing *in vivo*, we developed and validated protocols for RT-PCR and RNA extraction from fibroblasts, lymphoblasts, and lymphocytes. Sequencing of *TCOF1* transcripts from these three tissues from unrelated non-TCS individuals confirmed that the reported 3' site of exon 22 was used exclusively, thereby ruling out the possibility of alternate splicing of exon 22. Surprisingly, sequencing of lymphocyte RNA from the TCS patient with the 3381A>C mutation demonstrated skipping of exon 22 in entirety. Subsequent computational analysis with ESE Finder revealed two putative exonic splice enhancers, both of which are disrupted by this mutation. The loss of exon 22 is predicted to create a premature stop codon in exon 23 perhaps leading to nonsense mediated RNA decay of the mutated transcript. However, interrogation of a polymorphism in exon 2 showed approximately equal levels of transcripts from each *TCOF1* allele. Thus, on the basis of observed transcript stability we suspect that a truncated nonfunctional protein is produced from this allele. This expanded analysis of a patient with TCS identified the first putative splicing enhancer in *TCOF1* and demonstrates that complementing DNA and RNA analyses can reveal effects of sequence variations on gene function that are not predicted from genomic sequencing alone.

The Prevalence of GJB2 mutation in the baloochi population in Iran. *A. Naghavi¹, K. Kahrizi¹, J. Oveysi², M. Shakiba², H. Suraki Aliabadi³, Y. Riazalhosseini¹, N. Bazazzadegan¹, N. Mahdie¹, M. Mohseni¹, R.J.H. Smith⁴, H. Najmabadi¹.* 1) Genetics research center, Social welfare univ. , Tehran, Tehran, Iran; 2) Social Welfare & Rehabilitation Organization of Zahedan , Iran; 3) Zahedan University of Medical Sciences , Zahedan , Iran; 4) Molecular Otolaryngology Research Laboratories , Department of Otolaryngology , University of Iowa , Iowa City , IA ,USA.

Hereditary hearing impairment is a heterogeneous disability showing different pattern of inheritance and involving a multitude of different genes. Mutations in the GJB2 gene, especially the 35delG mutation, have been established as a major cause of inherited and sporadic non-syndromic deafness in various ethnic groups . The aim of our study was to determine the prevalence of GJB2 , 35delG mutation in the baloochi population in Iran . Fifty patients were examined by an extensive questionnaire to exclude syndromic forms and environmental causes of deafness. Informed consent was taken from all the patients in the study. Mutation screening of GJB2 was performed by allele-specific PCR for the detection of the 35delG mutation. The negative cases for 35delG mutation were screened by DHPLC and sequencing analysis. The 35delG mutation was not found in any of the patients. We identified 5 mutations in exon 2 (R127H, V153I, W24X, 167delT and a novel mutation, M93I) . Based on these data, the hot-spot mutations in Cx26 gene in baloochi population with non-syndromic hearing loss may be different from other ethnic groups in Iran.

A spectrum of epidermolysis bullosa phenotypes resulting from mutations in the plectin gene. *E. Pfindner, F. Rouan, J. Uitto.* Dept Dermatology & Cutaneous Biology and DebRA Molecular Diagnostics Laboratory, Thomas Jefferson Univ, Philadelphia, PA.

Epidermolysis bullosa (EB) is a group of heritable blistering diseases with extracutaneous manifestations. One subtype, EB with muscular dystrophy (EB-MD), is characterized by tissue separation within the basal keratinocytes just above the hemidesmosomes, accompanied by proximal muscle weakness that can develop during childhood (early-onset) or in the third decade of life (late-onset). Plectin is a component of the hemidesmosomal inner plaque and is also expressed in the muscle sarcolemma and the Z bands. A variety of mutations that result in premature termination codons in the plectin gene (PLEC1) have been identified in patients with EB-MD. Our laboratory has collected a cohort of 14 patients with EB-MD and identified mutations in PLEC1. In addition, two patients with EB with pyloric atresia (EB-PA) with neonatal lethality displayed epidermal tissue separation above the basement membrane and mutations in PLEC1 were identified. A comparative study of the patients examined in this laboratory and reported in the literature disclosed PLEC1 mutations in 27 patients, four of which have the lethal EB-PA phenotype and 20 with early-onset and three with late-onset EB-MD. In all 27 patients with plectin mutations skin blistering of variable severity was noted. In two of three late-onset EB-MD patients small in-frame insertions or deletions were identified on both alleles within the 4 integrin-binding domain of the protein. In the four EB-PA patients, the plectin mutations were located within the 4 integrin binding domain of the protein, resulted in STOP codons and predicted accelerated mRNA decay and absent protein. All of the mutations in the 23 early-onset EB-MD patients, without pyloric atresia, occurred outside of the 4 integrin-binding domain and resulted in STOP codons in the mRNA. The presence of mutations within the 4 integrin-binding domain attests to the functional significance of this portion of the protein and emphasizes its importance in interactions with 4 integrin in the development of the maturing fetal skin and gastrointestinal tract.

Hereditary Haemorrhagic Telangiectasia: ENG and ALK1 mutations in Dutch patients. *H.K. Ploos van Amstel¹, T.G.W. Letteboer¹, F.A.M. Hennekam¹, G. de Haas¹, E. Kamping¹, J.J. Mager², C.J.J. Westermann².* 1) Department of Medical Genetics, University Medical Center, Utrecht, The Netherlands; 2) St. Antonius Hospital, Nieuwegein, The Netherlands.

Hereditary Haemorrhagic Telangiectasia (HHT or Rendu-Osler-Weber disease (MIM 187300)) is an autosomal dominant disorder characterized by localized angiodysplasia. The resulting vascular lesions are direct arteriovenous connections without an intervening capillary bed. This can result in a range of malformations from smaller mucocutaneous telangiectases to large visceral arteriovenous malformations. The estimated prevalence in the Netherlands is 1:30,000. Mutations in at least two genes have been shown to be associated with the disease, endoglin (ENG on chromosome 9q34, HHT1) and ALK1 (ACVRL1 on chromosome 12q13, HHT2) respectively. To date, we have studied 127 probands with Rendu-Osler-Weber disease that fulfilled the Curaçoa criteria. A sequence analysis has been performed for both the ENG gene (exon 1-14) and the ALK1 gene (exon 2-10). Mutations were found in both genes: 56% of the probands had a mutation in the ENG gene, whereas 36% had a mutation in the ALK1 gene. No proband has been identified with deleterious mutations in both genes. The mutations detected were deletions, insertions, nonsense, missense and splice site mutations. Several mutations were found more frequently. For these, genealogical study showed a common ancestry that could be dated as far back as the 18th century. Currently, we are investigating the remaining 8% of the probands for gross gene abnormalities. These mutations will be missed using a sequence based approach. Organizing the clinical data, the genotype-phenotype relationship of the two patient groups with an endoglin respectively an ALK1 mutation shows that PAVM and CAVM are significantly more frequent in carriers of an ENG mutation. No significant difference for epistaxis exists between the two groups, although in HHT1 patients epistaxis starts earlier. The identification of carriers enables appropriate medical attention and the prevention of unnecessary clinical screening of non-carrier children in the family.

Evaluation of the GJB6 large deletion in the Iranian patients with autosomal recessive non-syndromic hearing

loss. *Y. Riazalhosseini*^{1, 2}, *C. Nishimura*³, *K. Kahrizi*¹, *A. Daneshi*⁴, *S. Arzhangi*¹, *M. Mohseni*¹, *N. Mahdieh*¹, *M. Avenarius*³, *RJH. Smith*³, *H. Najmabadi*¹. 1) Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Tehran, Iran; 2) Khatam University, Tehran, Iran; 3) Molecular Otolaryngology Research Laboratories, Department of Otolaryngology, University of Iowa, Iowa City, IA, USA; 4) Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran.

Hearing impairment affects 1 in 1000 newborns and about half of these babies, the loss has a genetic basis. Autosomal recessive non-syndromic sensorineural deafness (ARNSD) is the most common form of severe inherited hearing impairment. To date, at least 40 loci for ARNSD have been identified, indicating it as an extremely heterogeneous disorder. These loci are referred to as *DFNB* loci. Mutations in the gene that encodes the gap-junction protein connexin 26 (*GJB2*) at the *DFNB1* locus on chromosome 13q12 are the most common cause of ARNSD in many different populations. A second gap-junction gene, *GJB6*, also localizes to the *DFNB1* interval. Interestingly, the encoded protein connexin 30 is expressed in the same inner-ear structures as connexin 26 and both connexins are functionally related. The importance of *GJB6* to normal hearing has been confirmed by the identification of a large deletion involving the first two exons and a part of third exon of *GJB6* and a large region of the upstream sequence ((*GJB6*-D13S1830)) in persons with ARNSD. Homozygotes for this deletion and compound heterozygotes carrying (*GJB6*-D13S1830) and a deafness-causing allele variant of *GJB2* have severe-to-profound congenital deafness. To evaluate the importance of (*GJB6*-D13S1830) in the Iranian population, we screened 116 Iranian deaf probands with normal *GJB2* alleles in addition to 16 Iranian probands heterozygote for only one *GJB2* mutation for this deletion. Screening for (*GJB6*-D13S1830) was completed using PCR primers that amplify the breakpoint junction of this deletion. (*GJB6*-D13S1830) was not detected in our subjects, indicating that this mutation is not a common cause of deafness in Iran. Our finding suggests that (*GJB6*-D13S1830) is not wide-spread in the world.

Identification of Four Novel Pathogenic Mutations in Patients from Southern Italy with CADASIL. *C. Ungaro, T. Sprovieri, F.L. Conforti, A. Magariello, G. Di Palma, A. Patitucci, O. Gallo, A.L. Gabriele, G. Peluso, M. Muglia, R. Mazzei.* Institute of Neurological Sciences-CNR, Mangone, Cosenza, Italy.

Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL; MIM#125310) is a late-onset syndrome characterized by subcortical ischemic strokes, attacks of migraine with aura, and vascular dementia. Subcortical dementia, in all cases associated with pseudobulbar palsy, is the second commonest clinical manifestation of CADASIL. All individuals, both symptomatic and asymptomatic, have prominent signal abnormalities on brain Magnetic Resonance Imaging (MRI). CADASIL is caused by mutations in the Notch3 gene encoding a transmembrane receptor. The contribution of Notch3 mutations to the Italian population incidence of CADASIL was unknown. In the present study, twenty-five patients from 10 families of South Italy have been clinically examined and submitted to neuroimaging studies. Molecular investigation has been performed on 22 of 33 exons of the Notch3 gene encoding for the extracellular domain, containing the 34 Epidermal Growth Factor (EGF)-like repeats, by DHPLC and following direct sequence. Ten pathogenic mutations have been detected: two in the exon 3 (2x R90C), two in the exon 6 (2x R322C), four in the exon 8 (C428T, C428R, C440S, R449C), one in the exon 10 (G528C), and one in the exon 22 (R1231C). Three mutations in the exon 8 and one in the exon 10 are new. All these missense mutations involve gain or loss of a cysteine residue. Furthermore, we detect 14 different polymorphisms, 7 out of 14 were nonsense substitutions, 5 were amino acid changes, 2 intronic variations.

A Novel Allele of the β -Globin Gene Cluster Containing Both the $\beta^{3.7}$ and $\beta^{\text{anti4.2}}$ Crossover Junctions - Implications for Thalassemia Genotyping. W. Wang¹, A.Y.Y. Chan², E.S.K. Ma², S.S. Chong^{1,3}. 1) Pediatrics, Ob/Gyn, Lab Medicine, National Univ Singapore & Hospital, SINGAPORE; 2) Pathology, Univ Hong Kong & Queen Mary Hospital, Hong Kong, PRC; 3) Pediatrics, Gyn/Ob, Institute of Genetic Medicine, Johns Hopkins Medicine, USA.

Misalignment and unequal crossover between the homologous XYZ boxes of the β -globin gene cluster results in single β -globin gene deletions (-) and reciprocal β -globin gene triplications (). During screening of patients for β -globin gene deletions by Southern analysis, an anomalous ~20 kb *Bgl*III - / -globin fragment was detected in a β -thalassemia major patient (Cd 41/42 / Cd 41/42) and an unrelated β -thalassemia carrier (-28 /). Subsequent β -globin multiplex-PCR analysis indicated the presence of both the $\beta^{3.7}$ and $\beta^{\text{anti4.2}}$ alleles in both patients. A *bona fide* compound heterozygous $\beta^{3.7} / \beta^{\text{anti4.2}}$ sample showed the same PCR results but not the anomalous ~20kb fragment. Parental DNAs were available for the thalassemia major patient. Southern and PCR analyses revealed a normal paternal β -globin genotype, but a maternal result identical to the patient, thus showing that the patient cannot be compound heterozygous for $\beta^{3.7}$ and $\beta^{\text{anti4.2}}$. We hypothesized that the patient, her carrier mother, and the unrelated carrier are heterozygous for a novel rearranged β -globin cluster arising from two sequential unequal crossover events. This dual recombinant allele contains both the $\beta^{3.7}$ and $\beta^{\text{anti4.2}}$ unequal crossover junctions, resulting in no net gain/loss of β -globin genes. We devised a large fragment nested PCR strategy to test this hypothesis. Predicted fragments of the expected sizes were observed only in the three individuals, thus confirming the presence of the novel allele in them. The existence of this dual recombinant allele dictates caution in the interpretation of PCR-based β -globin genotyping results. DNA samples positive for both the $\beta^{3.7}$ and $\beta^{\text{anti4.2}}$ junction fragments by PCR analysis should be confirmed by Southern or large fragment nested PCR analysis in order to discriminate between the compound heterozygous $\beta^{3.7} / \beta^{\text{anti4.2}}$ state and heterozygosity for the novel " $\beta^{3.7+\text{anti4.2}}$ " allele.

Increasing the Jagged1 Mutation Detection Rate in Alagille Syndrome. *D.M. Warthen, E.C. Moore, B.M. Kamath, S. Yehuda, J.J.D. Morrissette, N.B. Spinner.* Div of Human Genetics, Dept of Pediatrics, The Children's Hospital of Philadelphia and The University of Penn School of Medicine.

Alagille syndrome (AGS) is caused by mutations of Jagged1 (JAG1). Studies from multiple laboratories have demonstrated JAG1 mutations in 60-70% of patients with AGS. Screening has been performed using single strand conformation polymorphism (SSCP) or denaturing high-pressure liquid chromatography (DHPLC). The inability to detect mutations in the remaining patients has been thought to be due to technical limitations in screening this 26-exon gene, though it raises the possibility that there is another locus for AGS. To rule out the presence of a second locus, we developed an aggressive screening protocol. We studied 233 patients with AGS. Initial screening was done by SSCP (190 patients) or conformation sensitive gel electrophoresis (CSGE) (43 patients). If no mutation was identified, sequencing of cDNA or genomic DNA was carried out. Of the 190 patients screened using SSCP, JAG1 mutations were identified in 123 (65%) individuals. Twenty-six of the 67 patients in whom no mutations were identified have had further investigations (CSGE, sequencing) and mutations were identified in 17/26. Therefore, of the patients completely studied, 140/149 had mutations detected. Of 43 patients initially screened using CSGE, mutations were identified in 29(67%). Nine of the 14 patients unshifted on CSGE underwent sequencing and 6 mutations were found (35/38). In total, 187/233 patients completed a sequential approach to mutation identification, with mutations found in 174/187 (93%) patients. To further study the patients in whom no mutation has been identified, we developed an RNA based test to look for instability of a mutant RNA, which would suggest nonsense-mediated decay. cDNA is analyzed for the presence of known polymorphisms when cells are grown in the presence and absence of cycloheximide (which stabilizes mutant RNA). To date, we have studied 4 patients and found evidence for one unstable JAG1 allele in 2/4, further increasing our suspected mutation rate. This higher rate of mutation identification in AGS (93%) reduces the likelihood that another locus for this condition exists.

Duplications within the DMD gene. *S.J. White¹, A. Aartsma-Rus¹, T. Lalic², K. Flanigan³, I. Ginjaar¹, G.J.B. van Ommen¹, M.H. Breuning¹, J.T. den Dunnen¹.* 1) Center for Human & Clinical Genetics, Leiden University Medical Center, The Netherlands; 2) Laboratory for Medical Genetics, Mother and Child Health Institute of Serbia, Serbia and Montenegro; 3) Departments of Neurology and Human Genetics, University of Utah, Salt Lake City, Utah, USA.

The Duchenne Muscular Dystrophy (DMD) gene is the largest known, spanning ~2.4 Mb of genomic sequence on Xp21. Mutations in the gene cause DMD and BMD, with deletions and duplications of one or more exons being found in ~70% of cases. Deletions in males can be easily detected, whereas duplications require a quantitative method of analysis. Using Multiplex Amplifiable Probe Hybridisation (MAPH) and Multiplex Ligation-dependent Probe Amplification (MLPA) we rescreened a cohort of patients with hitherto undetected mutations and found over 40 duplications. The majority of cases contain a simple contiguous duplication, however we also detected several non-contiguous duplications, with one including a triplication as well. In such instances the 3' end of the gene is often affected, which is a region not usually screened by multiplex PCR. These mutations would therefore go undetected, whilst potentially disturbing the reading frame of the mRNA. This emphasizes the importance of screening the entire gene for rearrangements. Overall, there is a significant difference in the distribution of deletions and duplications. Whilst the major deletion hot spot is localised around exons 45-55, more than 50% of the duplications are at the 5' end of the gene. A more detailed comparison of the regions affected showed that a duplication of exon 2 only was the single most common duplication found, whereas the reciprocal deletion has never been described. This could be due to structural or mechanistic restraints, or it may be that a deletion within this region is not compatible with life. Initial mapping and sequencing of duplication junctions shows that all exon 2 duplications were of a different size, suggesting that there is no specific hotspot for recombination within either intron 1 or intron 2. A better characterization of these rearrangements should increase our understanding of the molecular mechanisms behind these events.

Hemizyosity for *NPHS1* (nephrin) mutations associated with congenital nephrosis, (Finnish type). C.A.

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Congenital nephrosis (Finnish type, OMIM 256300) is an autosomal recessive condition caused by mutations in the *NPHS1* gene that codes for nephrin. A 4-month-old African-American female with edema and proteinuria had renal biopsy findings consistent with congenital nephrosis, Finnish type. Both parents are healthy. Consanguinity and non-paternity were denied. DNA testing of the patient revealed apparent homozygosity for eight single nucleotide transitions or transversions: T1110C, A1926G, GIVS7 + 6A, AIVS7 + 26T, C2289T, T2728C, T2971G, and CIVS24 + 36T. T2728C should produce a Ser910Pro amino acid sequence change, and T2971G should produce a Val991Leu change. Her mother was heterozygous for the T1110C, A1926G, GIVS7 + 6A, AIVS7 + 26T, C2289T, T2728C and T2971G mutations, and homozygous for the CIVS24 + 36T mutation. The patient's father was homozygous for five single nucleotide transitions or transversions: CIVS1-5G, AIVS3 + 61, C1320T, C2289T, and CIVS24 + 36. The patient was apparently homozygous for two mutations that would result in amino acid sequence changes although neither of these mutations was present in her father. She was also apparently homozygous for four other silent mutations that were not present in her father. Neither of the missense mutations has been reported previously, and their significance is unknown.

The simplest explanation for these findings is the father has a deletion that spans from at least nt1110 to nt2971. Inheritance of the deleted chromosome from the father would allow the detection of apparent homozygosity in the daughter of alleles that are heterozygous in her mother. If the only intact gene produces a protein with two amino acid changes this may affect the function of nephrin and cause disease. This mechanism of disease has not been reported previously in this disease. Mutations associated with this disease have not been reported previously in this ethnic group. Molecular studies to confirm the mechanism are being pursued.

Screening of the Sperm Nuclear Protein Genes in a Population of Infertile Males. *V.W. Aoki, G.L. Christensen, D.T. Carrell.* IVF and Andrology Laboratories, University of Utah, SLC, UT.

Introduction: During spermatogenesis, spermatid chromatin undergoes substantial compaction. This process occurs by the replacement of the somatic histones with the transition nuclear proteins and, subsequently, the protamine proteins. These sperm nuclear proteins are often abnormally expressed in cases of severe male-factor infertility. The objective of this study was to evaluate the sperm nuclear protein genes for clinically relevant mutations in a population of infertile males.

Methods: A total of 96 males presenting with severe male factor infertility, and 48 fertile controls were enrolled in the study after informed consent. Genomic DNA was isolated and PCR primers were developed to amplify the promoter region and exons of transition protein 1, transition protein 2, protamine 1 and protamine 2. Internal primers were designed to allow sequencing in both the forward and reverse directions. Sequence trace files were assembled and evaluated using Phrap, Phred, and Consed software.

Results: A total of six different heterozygous polymorphisms were identified in the four genes studied. Three were found in intronic regions and three in the exons. Two resulted in amino acid changes. The first change, identified in two infertile patients and one fertile control, converted an arginine residue to lysine in exon one of protamine 1. The second, identified in a single infertile patient, resulted in a conversion of lysine to glutamate in exon one of transition protein 1.

Conclusions: At least one of the identified polymorphisms resulting in an amino acid change may contribute to the infertility of the affected individual. The data also suggest that mutations within the nuclear proteins are rare and not a common cause of male factor infertility. Our findings suggest that other factors are responsible for the abnormal nuclear protein expression observed in many infertile males.

Mutation screening of COH1 and BAFME on 8q22-q24.1. *S. Asakawa¹, K. Miyamoto², A. Shimizu¹, S. Yamazaki¹, S.K. Ishikawa¹, T. Sasaki¹, J. Kudoh¹, H. Yamagata², Y. Tabara², S. Minoshima³, I. Kondo², N. Shimizu¹.* 1) Department of Molecular Biology, Keio University School of Medicine, Tokyo, Japan; 2) Department of Hygiene, Ehime University School of Medicine, Ehime, Japan; 3) Photon Medical Research Center, Hamamatsu University School of Medicine, Hamamatsu, Japan.

We have sequenced the 20Mb-genomic region of 8q22-24.1. We also made detailed annotation on the 51.6 Mb-region covering 8q22-qter, where we found 260 protein coding genes and 179 putative non-coding RNA genes or pseudogenes. Several genetic diseases including Cohen syndrome and benign adult familial myoclonus epilepsy (BAFME) are mapped in this region. The Cohen syndrome is an autosomal recessive disorder characterized by psychomotor retardation and microcephaly, distinct facial appearance, childhood hypotonia and joint hyperextensibility etinocholoidal dystrophy and myopia, and periods of isolated leucopenia. In addition to the authentic Cohen syndrome (Finnish Type), a Cohen-like syndrome (Israeli type) was also reported. Recently, a causative gene of Cohen syndrome, COH1, was identified, so we searched COH1 mutation in two Japanese Cohen syndrome families with Finish type and Israeli type. We found a mutation in the Finish type patient. BAFME is inherited in an autosomal dominant manner and is characterized by adult-onset tremulous finger movement, myoclonus, epileptic seizures, and a non-progressive course. BAFME is recognized mainly in Japanese families and the causative locus was mapped on 8q24.1. We have performed mutation screening against the coding regions of all genes in the causative region, but we found no mutations in 53 genes. Therefore, we started to mutation search in the regulatory regions of these genes and the non-coding RNA genes found in the region. We will present the results of our comprehensive mutation screening.

Identification and characterization of the breakpoint region in a Japanese Sotos syndrome patient with a common microdeletion. *R. Visser*^{1,2,3,4,5}, *N. Niikawa*^{1,4}, *N. Matsumoto*^{3,4}. 1) Department of Human Genetics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; 2) Department of Pediatrics, Leiden University Medical Center, Leiden, The Netherlands; 3) Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan; 4) CREST, Japan Science and Technology Corporation, Kawaguchi, Japan; 5) International Consortium for Medical Care of Hibakusha and Radiation Life Science, The 21st Century COE (Center of Excellence), Nagasaki, Japan.

Sotos syndrome (SoS) (OMIM #117550) is a childhood overgrowth syndrome characterized by pre- and postnatal overgrowth, distinctive craniofacial features, and various degrees of developmental delay. Aberrations of the *NSDI* gene, which include intragenic mutations or microdeletions of *NSDI* and adjacent genes, account for approximately 60-70% of the SoS patients. A striking difference in microdeletion and mutation prevalence is reported between Japanese and non-Japanese SoS patients, with microdeletions being far more prevalent in the Japanese SoS patient population. Low copy repeats have been identified harboring the deletion breakpoints in SoS. Abnormal genomic rearrangements between these areas of high homology, so called Non Allelic Homologous Recombination (NAHR), are hypothesized to be underlying mechanism causing the microdeletions in SoS. PFGE and hybrid cell lines are the techniques commonly used for characterization of the junction fragments in genomic disorders. We here report the construction of a BAC library of the genome of a Japanese SoS patient with a common microdeletion. PCR based library screening enabled us to identify the clone containing the breakpoint region as well as other clones of the flanking low copy repeats. Currently we are developing a screening system for the identification of a similar breakpoint region in other Japanese SoS patients. The identification and characterization of the breakpoint region will give more insight in the underlying mechanism of microdeletions in SoS.

New mutations in KIAA1985 gene implicated in a demyelinating form of autosomal recessive Charcot Marie Tooth (CMT4C) disease. *H. Azzedine¹, N. Ravise¹, A. Gabrels-Festen², M. Tazir³, N. Birouk⁴, D. Grid⁵, A. Brice^{1, 6}, J. Senderek⁷, E. LeGuern^{1, 6}, the GIS-orphan diseases institute network on autosomal recessive form of Charcot-Marie-Tooth disease⁸.* 1) INSERM U289-NEB, Hpital de la Salpatriere, Paris, France; 2) Institute of Neurology, University Medical Centre Nijmegen, Nijmegen, the Netherlands; 3) Service de Neurologie, CHU Mustapha, Algiers, Algeria; 4) Service de Neurologie, Hpital des Spcialits, Rabat, Morocco; 5) AFM-BTR, Gnthon, Paris, France; 6) Neurogenetics laboratory, Pitié-Salpêtrière Hospital, AP-HP, Paris, FRA; 7) Departments of Human Genetics and Neuropathology, Aachen University of Technology, Aachen, Germany; 8) the GIS-orphan diseases institute network on autosomal recessive form of Charcot-Marie-Tooth disease.

The CMT is a pathologically and genetically heterogeneous group of hereditary motor and sensory neuropathies characterized by slowly progressive weakness and atrophy, primarily in peroneal and distal leg muscles. Two major types have been distinguished: demyelinating or axonal form. Several loci (30) and various modes of inheritance were described: autosomal dominant, X linked and autosomal recessive (ARCMT). The demyelinating form of CMT is characterized by reduced nerve-conduction velocities in the medium nerve (MNCVs <38 m/s), segmental demyelination and remyelination, and onion bulb formation in nerve biopsy. The ARCMT type 4C (CMT4C) is a childhood-onset demyelinating form of hereditary motor and sensory neuropathy often associated with an early-onset scoliosis. twelve families from different geographical and ethnical origins with linkage to 5q23-q33 locus where screened for the KIAA1985 gene (Senderek et al 2003) in order to identify the responsible mutations. Direct sequencing on affected individuals identified 9 new mutations localised in different portions of the gene. One among them was recurrent in these families, with haplotype reconstructions suggesting a founder effect. This series of 31 patients allow to define the phenotype associated with mutation in KIAA1985. SnapShot experiments (Applied Biosystems) are in progress to confirm these data.

A novel LIM2 mutation associated with autosomal recessive non-congenital cataract. *G.D. Billingsley¹, A.V. Levin^{2, 3}, S. Herd¹, R. Sidhu¹, E. Héon^{1, 2, 3}.* 1) Genetics and Genomic Biology, The Hospital for Sick Children Research Institute, Toronto, ON, Canada; 2) Dept of Ophthalmology and Vision Sciences, Hospital for Sick Children, Toronto, ON, Canada; 3) University of Toronto, Department of Ophthalmology and Vision Sciences, Toronto, Ontario, Canada.

Cataracts constitute a leading cause of blindness worldwide and the mechanisms of lens opacification remain unclear. Approximately one-third of all congenital cataracts are familial and recent studies suggest that ~50 % of age-related cataracts could be accounted for by Mendelian inheritance. In contrast to the large number of genes and loci (at least 20) identified in human autosomal dominant cataract, only three genes, CRYAA, LIM2, GCNT2 and two loci with as yet unidentified genes have been implicated in AR cataract. We have studied a consanguineous Lebanese family in which two sibs had early onset bilateral cataract. STRP markers for the previously published AR loci (3p21.1 - p21.3, 9q13-q22, 19q13.4, 21q22.3) were chosen for genotyping. Markers in a 2 cM region flanking the LIM2 locus (19q13.4) were homozygous in the two affected sibs. All other AR loci were excluded by haplotype analysis. Sequence of the LIM2 coding exons (exon 2-5) was normal. A novel homozygous sequence change (tg) was observed 33 base pairs 3' of the stop codon. This nucleotide is part of a 6 base pair segment highly conserved in mouse, rat, bovine and human. The sequence change removes a MseI restriction enzyme site and was shown to cosegregate with the affected status in the family. This change was not seen in one hundred control chromosomes which were screened by means of the restriction enzyme digestion, but only the wild-type variant was found. The molecular characterization of AR cataract may contribute to the better understanding of the complex cascade of events modulating lens opacification in the more common age-related cataract. Grant Support: Canadian Institute for Health Research.

Haplotypes of the TAS2R38 Bitter Taste Receptor Link PTC and PROP Psychophysical Functions With *in vitro* Expression Sensitivity. P.A.S Breslin¹, D.R. Reed¹, C.D. Tharp¹, U.K. Kim², D. Drayna², B. Bufe³, W. Meyerhof³, C.

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Most human populations may be subdivided according to whether they are sensitive to bitterness from PTC. Recently a gene, TAS2R38 on Chr 7 was associated with PTC recognition thresholds in humans (Kim et al., Science 2003). Presently, we screened Kim et al's sample population (and others) for recognition thresholds and suprathreshold concentration-intensity functions for PTC & PROP. We simultaneously tested three individuals with specific TAS2R38 haplotypes at amino acid positions 49, 262, 296 who are homozygous for the AVI (non-taster), PAV (taster), and the rarer recombinant AAI haplotype. For each of these three, we measured recognition thresholds and psychometric functions with the method of constant stimuli to determine their detection thresholds to PTC & PROP. These functions enabled direct comparison between the humans perceptual thresholds and the *in vitro* thresholds of their haplotypes as determined by an HEK293 cellular expression system in which their TAS2R38 genes were cloned and tested. We found that homozygous AVI and PAV subjects' PTC psychophysical measures were highly correlated with haplotype and had virtually no overlap between these two groups. Both the heterozygous subjects and the AAI subject had intermediate sensitivity. PROP intensity ratings were correlated with haplotype at perithreshold and low intensity concentrations but not at high concentrations. PTC sensitivities based upon psychometric functions were very similar to *in vitro* measures with the subjects expressed TAS2R38 proteins. Thus, a bottom-up approach to understanding genetic contributions to perception can successfully explain individual differences in bitter taste. This research was funded in part by NIH DC02995 and DC004698 (breslin@monell.org).

Polymorphisms at PRX gene causing Charcot-Marie-Tooth Phenotype. *A. Cornier¹, J. Acevedo², S. Carlo¹, E. Suarez¹, N. Arciniegas³, I. Flores⁴.* 1) Genetic Division, Ponce School Medicine, Ponce, PR; 2) Department of Nursing, Univ of Puerto Rico at Arecibo, Arecibo, PR; 3) Department of Pediatrics, Ponce School of Medicine, Ponce, PR; 4) Department of Microbiology, Ponce School of Medicine, Ponce, PR.

Charcot-Marie-Tooth (CMT) (MIM# 118220) is a polyneuropathy syndrome that presents a heterogeneous group of peripheral nerve disorders. Clinical manifestations vary and can be classified in two large groups: CMT1 which represents a symmetrical demyelinating disease that presents earlier in life, usually of autosomal dominant inheritance whereas CMT2, autosomal dominant as well, manifests later in life. We are presenting a case of a 14 years old female (KG) who presents with clinical manifestations consistent with CMT1 including hammer toes, gait disturbances, distal muscle weakness, cold intolerance and diminished deep tendon reflexes. DNA mutation analysis for CMT showed an *ERG2* sequence alteration consisting of an A C transversion at position 1086 that do not codified for an amino acid change. *PRX* allele one had a sequence alteration consisting of a C A transversion at position 619 that codified for a leucine to methionine change in her other allele. Interestingly, she presents additional polymorphisms at her other *PRX* allele consisting of an Ala882Val, and Pro885Pro. Although when the clinical meaning of these polymorphisms are not determined the DNA sequence in this patient is predicted to change the amino acid sequence of the protein and thus may altered the protein function. Nerve conduction studies confirm clinical findings showing mild prolonged median and ulnar DMLs with reduced median, ulnar and tibial motor amplitudes with an absent peroneal motor response. Sensory amplitudes were reduced for the radial, median and ulnar nerves with an absent serial sensory response. We believe that these polymorphisms do translate to clinical manifestations in our patient consisting of a CMT1 mild to moderate presentation. Genetic analysis of *PMP-22*, *Cx32*, *MPZ*, and *NF-L* genes was performed and no sequence alteration detected.

Search for modifier genes of cystic fibrosis: candidate gene approach. R. Dorfman¹, A. Sandford², D. Markiewicz¹, A. Master¹, G. Deng¹, M. Corey¹, X.-W. Yuan¹, M. Tan¹, F. Lin¹, D. Frangolias², P.D. Paré², L.-C. Tsui^{3,1}, P. Durie¹, J. Zielenski¹, *Canadian CF Clinics*. 1) Genetics and Genomic Biology, Hospital for Sick Children, Toronto, ON, Canada; 2) University of British Columbia, Vancouver, BC, Canada; 3) University of Hong Kong, Hong Kong.

Clinical severity of cystic fibrosis (CF) is determined both by mutations in cystic fibrosis transmembrane conductance regulator (CFTR) as well as variations in other genes (CF modifiers). One of the approaches to identifying CF modifiers is analysis of candidate genes selected on the basis of their relevance to various aspects of CF disease. This approach is used in the Canadian CF Modifier Gene Project recently launched to map and identify genes influencing severity of CF. We report the preliminary results of association analyses of genes tested in the study as potential CF modifiers. In total 57 genes were selected for the study. The criteria of selection were based on their functional relevance to the pathophysiology of CF including antibacterial defense, innate and adaptive immune response, mucociliary clearance, tissue repair, protein folding and lung morphogenesis. We selected 89 intragenic polymorphisms (mainly SNPs) for genotyping a Canadian cohort of CF patients (N=513) and families (N=330). Association between polymorphic markers and clinical variables was evaluated either by ANOVA for CF patients or Family-Based Association Tests (FBAT) for CF families. Partial results were obtained for 48 intragenic markers in CF patients and/or CF families. Our preliminary analysis of the genotypic and clinical data identified 10 markers corresponding to 9 genes with statistical significance for specific clinical variables. Four of them correspond to genes that have been previously implicated as CF modifiers. They are genes encoding surfactant proteins (SP-A and D), transforming growth factor beta (TGF) and defensin beta (DEF). As we ascertain additional CF families, we continue association analysis of these candidate genes in order to verify our findings in the larger study population. (Supported by Genome Canada and Canadian CF Foundation).

Implication of a novel sequence change in *BBS6* resulting in a mild BBS phenotype. *W. Ferrini*^{1,3}, *N. Noordeh*^{1,2}, *S. Herd*¹, *A. Levin*³, *E. Héon*^{1,2,3}. 1) The Genetics and Genomic Biology Program, The Hospital for Sick Children Research Institute, Toronto, Ontario, Canada; 2) The Institute of Medical Sciences, University of Toronto, Ontario, Canada; 3) Department of Ophthalmology and Vision Sciences, The Hospital for Sick Children, Toronto, Ontario, Canada.

Purpose: Bardet-Biedl syndrome (BBS) is an autosomal recessive multi-systemic disorder. BBS ultimately leads to blindness due to severe retinitis pigmentosa. BBS is genetically heterogeneous with 8 loci identified. Mutations in *BBS6* are proposed to account for a minority of cases (4-5%) (Katsanis et al., 2001). We investigated the role of *BBS6* (MIM 604896) in an ethnically diverse patient population affected with BBS.

Methods: Twenty-eight patients diagnosed with BBS were recruited through The Hospital for Sick Children, Canada. All 4 coding exons and the flanking introns of *BBS6* were screened for sequence changes using direct cycle sequencing.

Results: A novel homozygous missense sequence change was identified at amino acid position 99 (C99R) in an 11 year old boy. The phenotype of this patient included good school performance in grade 6, body weight in the 95th percentile, post axial polydactyly of 4 limbs, and mild kidney abnormalities with normal function. Visual acuity was measured at 20/30 in both eyes. Electroretinography showed non-recordable rod responses but recordable rod-cone and cone function. His peripheral field was of 90 degrees horizontally. No other systemic features were documented.

Conclusion: Here we report a novel sequence change in *BBS6* resulting in a mild phenotype of BBS. The cysteine residue and the surrounding area are conserved between human, mouse and rat orthologues.

Alpha-synuclein gene multiplications are responsible for familial Parkinson disease. P. Ibanez¹, E. Lohmann^{1, 3}, B. Debarges¹, F. Durif⁴, A. Destee⁵, AM. Bonnet³, S. Laine¹, Y. Agid^{1,3}, A. Durr^{1,2}, A. Brice^{1,2,3}, French Parkinson's Disease Genetics Study Group. 1) INSERM U289, Hopital de la Salpetriere, Paris, France; 2) Departement de genetique, cytogenetique et embryologie, Hopital de la Salpetriere, Paris, France; 3) Federation de Neurologie, Hopital de la Salpetriere, Paris, France; 4) Service de Neurologie, CHU de Clermont-Ferrand, France; 5) Service de Neurologie, CHU de Lille, France.

A growing number of responsible loci/genes have been identified in familial forms of Parkinson disease (PD). The alpha-synuclein gene (PARK1) has been implicated in rare autosomal dominant forms caused by missense mutations or gene triplications and duplications. The aim of this study was to determine whether multiplications of the alpha-synuclein gene are responsible for a subset of familial forms of PD. For this purpose we screened 222 individuals from families with dominant PD, mostly from French Caucasian origin, for alpha-synuclein gene multiplications by semi-quantitative multiplex PCR. Cases with a duplication or triplication were further validated using newly developed microsatellite markers spanning the alpha-synuclein gene and flanking regions. We found 4 patients with a heterozygous duplication and one with a triplication. In the remaining 217 patients normal peak height ratios were detected. The phenotype in duplicated patients was indistinguishable from idiopathic PD but, as reported in recently reported cases, the patient with triplication of the synuclein gene presented atypical features and a more severe course of the disease. In addition to a dopa-responsive Parkinson syndrome with onset at age 52, the patient was demented after a disease duration of 6 years (MMS 6/30). He had swallowing difficulties and died at age 65. Taken together with previously reported results, this data strongly suggest that the severity of the phenotype increases with the number of copies of the gene. The identification of alpha-synuclein gene duplication in 4 independent families and triplication in one out of 222 with dominant PD indicates that mutations in the alpha-synuclein gene are more frequent than previously thought.

Spectrum of UGT1A1 mutations in Crigler-Najjar (CN) syndrome patients: identification of thirteen novel alleles and genotype-phenotype correlation. A. Iolascon¹, V. Servedio², M. d'Apolito², N. Maiorano², B. Minuti³, L. Boschetto¹, S. Ronchi⁴, F. Torricelli³. 1) CEINGE, Napoli, Napoli, Italy; 2) Molecular Medicine, University of Foggia; 3) U.O. Citogenetica, Az. Osp. Careggi, Firenze; 4) Ospedali Infermi, Rimini.

Crigler-Najjar syndrome types 1 and 2 (CN1,CN2) are inherited as autosomal recessive conditions and are characterized by severe non-haemolytic unconjugated hyperbilirubinaemia. CN1 is the most severe form, associated to the absence of UGT1A1 activity. A milder variant of this disorder CN2 presents incomplete deficiency of hepatic UGT1A1 activity. The causative gene, UGT1A1, on chromosome 2q37, encode for UGT isoenzyme 1A1. The analysis allows us to identify in 31 unrelated Crigler-Najjar (CN) syndrome patients 22 mutations, 13 of which were novel, including one nonsense mutation, two altered typical splice sites, one single amino acid deletion and none missense mutations. We report the first case of de novo mutation in a boy with CN2. We also tried to discern any possible correlation of structure-function of UGT1A1. Several novel missense mutations localize in critical domain of UGT1A1 enzyme. In addition, the evaluation of Gilbert-type promoter of UGT1A1 in Crigler-Najjar syndrome patients was performed. UGT1A1 mutations produced different phenotype resulting from the association with the polymorphism of the promoter region. The presence of at least of one TA7 allele in association with structural mutations of the UGT1A1 produces a more severe phenotype. For the 3 patients which have unique heterozygous missense mutations and a TATA-box insertion we detected the simultaneous presence of trans A(TA)₇TAA. This study represents the molecular characterization of the largest cohort of Italian Crigler-Najjar Gilbert syndromes patients studied so far; increase the mutational spectrum of UGT1A1 allelic variants worldwide; provide a new insight useful for clinical diagnosis and genetic counselling. Present study suggests that initial screening of any new Italian seeking Crigler-Najjar Gilbert syndromes type I should start by seeking for the common c.877T>A 878-890del that seems to be the most prevalent in Italian population.

Mutation analysis and regulating region characterization of CACNA1A gene coding for P/Q voltage-gated calcium channel 1A subunit. *C. Jodice¹, S. Albertosi¹, E. Mantuano², L. Veneziano², S. Guida², S. Lagnese¹, D. Pesce¹, M. Frontali².* 1) Department of Biology, "Tor Vergata" University, Rome, Italy; 2) Institute of Neurobiology and Molecular Medicine CNR, Rome, Italy.

The CACNA1A gene, coding for the 1A-subunit (Cav2.1) of voltage-gated calcium channel type P/Q is responsible for Episodic Ataxia type 2 (EA2), Familial Hemiplegic Migraine (FHM) and Spinocerebellar Ataxia type 6 (SCA6). Several mutations causing these diseases have been described. In addition, families segregating for the disease with the CACNA1A, but not showing any mutations in the coding region, have also been found. Thirty-four patients with typical EA2, and 8 with cerebellar ataxia of unknown genetic type, are screened for CACNA1A gene mutations. Four new Cav2.1 missense or non-truncating mutations and 1 truncating mutation have been detected. From an analysis of the localization of the non-truncating mutations together with those reported in literature it is further confirmed that: 1) they tend to be preferentially located in specific protein regions, namely S5-S6 linkers and their borders; 2) their associated clinical phenotype suggests a higher age at onset and a lower frequency of mental retardation. Families segregating for the disease with the CACNA1A markers, but not showing mutations of the coding region, suggest a further characterization of the gene. A new 3terminal exon, namely 48B, has been characterized. An alternative splice site about 300 bp upstream from 5end of exon 48 creates a so far unknown isoform similar to BI-1(V2.V3) but with a longer exon 48. Results from bioinformatic analysis show that the two isoforms with the alternatively spliced exon 48 have at least two different polyadenylation signal candidates. Since preliminary results suggest that mRNAs of this gene have dendritic localization and mRNA transport studies show a clear implication of the 3 tail in subcellular localization, these exons are being characterized both for the alternative polyadenylation signal usage and transport and for mutations causing disease in EA2 and cerebellar ataxia patients. Supported by grants MIUR cofin 2003 to CJ and FIRB2001, RBNE01XMP4-008, FISR2000 to MF.

Identification of a novel LAMB3 mutation causing Junctional Epidermolysis bullosa type Herlitz (JEB-H). *F.MK. Jonsson¹, T. Gedde-Dahl Jr^{2,3}, E-L. Stattin¹, G. Holmgren¹, J. Jonasson¹.* 1) Clinical Genetics, University Hospital of Ume, Ume, Sweden; 2) Institute of Forensic Medicine, University of Oslo, Oslo, Norway; 3) Dermatological DNA Laboratory, Department of Dermatology, Rikshospitalet University Hospital, Oslo, Norway.

Junctional Epidermolysis bullosa type Herlitz (JEB-H) is an autosomal recessive disorder causing severe blistering of skin and mucosa. The carrier frequency in Northern Sweden is estimated to 1/50. In Scandinavia, along with a R635X founder mutation in the laminin 5 gene LAMB3, families with apparent compound heterozygosity have also been identified.

Aiming to identify this unknown mutation(s), haplotype analysis of the LAMB3 gene region on chromosome 1q32 was undertaken in six North-Scandinavian JEB-H families. Haplotype analysis using 9 microsatellite markers covering approximately 32 cM region revealed identical 1q32 haplotypes for all six families, including one Norwegian family where the index patient was homozygous. Subsequent mutation screening of the LAMB3 gene identified a 1 bp deletion in exon 19, 3024 delT, resulting in a downstream premature termination codon. All alleles with the identified 1q32 haplotype could also be confirmed to carry the 3024delT mutation.

This mutation has previously been reported in a large Philadelphia kindred of unknown origin. Interestingly, this patient exhibited a non-lethal form of JEB and was reported to be a compound heterozygote with the genotype 3024delT/unknown mutation.

In conclusion, we here demonstrate that the 3024delT LAMB3 mutation results in the lethal type Herlitz form of JEB, when present either in combination with the R635X mutation, or in its homozygous state. Therefore, molecular testing for the 3024delT LAMB3 mutation is of significant value for prenatal diagnostics and genetic counseling in JEB-H families, especially in kindreds of Scandinavian origin.

Analysis of most common mutations H1069Q, R778G and R778L in Wilson disease patients from North West

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Wilson Disease (WD) is an autosomal recessive disorder of copper metabolism with a worldwide prevalence. The disorder manifests itself chronic liver disease and/or neurological impairment. The H1069Q point mutation in exon-14 of ATP7B is the most common mutation in Northern, Eastern, or Central European patients of WD. The other most common mutations R778L and R778G are present in population of Asian countries like Japan, Korea, china and Taiwan. The prime aim of this study was to investigate the frequency of most common mutations for assiduity of genetic testing in our population. A total of 57 patients with WD from 55 families residing in the North West states of India were examined. A seminested Polymerase Chain Reaction (PCR) based restriction fragment length polymorphism (RFLP) technique was used to detect the H1069Q mutation, while characterization of R778L and R778G mutations in exon-8 of ATP7B were carried out by using single strand conformational polymorphism (SSCP) and direct DNA sequencing. The data of our study are compatible with the hypothesis of the absence of H1069Q in Asian population, while exceptionally, the R778L and & R778G mutations were also absent in WD patients of North West India. Our data have also proved that the mutation in ATP7B tends to occur in a population- specific manner.

***NIPBL* Mutational Analysis in 120 Individuals with Cornelia de Lange Syndrome and Evaluation of Genotype-Phenotype Correlations.** I.D. Krantz¹, L.A. Gillis^{1, 2}, J. McCallum¹, M. Kaur¹, C. DeScipio¹, D. Yaeger¹, A. Mariani¹, A. Kline³, H. Li⁴, M. Devoto^{4, 5}, L.G. Jackson^{1, 6}. 1) Division of Human Genetics and Molecular Biology, The Childrens Hospital of Philadelphia and The University of Pennsylvania School of Medicine, Philadelphia, PA; 2) Divisions of Gastroenterology and Genetics, The Vanderbilt University Medical Center, Nashville, TN; 3) The Harvey Institute of Human Genetics, Baltimore, MD; 4) Nemours Childrens Clinic, Wilmington, DE; 5) Department of Biology, Oncology, and Genetics, University of Genoa, Italy; 6) The Division of Obstetrics and Gynecology, Drexel University School of Medicine, Philadelphia, Pennsylvania.

The Cornelia de Lange syndrome (CdLS [MIM #122470]) is a multisystem developmental disorder characterized by facial dysmorphism, upper extremity malformations, hirsutism, cardiac defects, growth and cognitive retardation, and gastrointestinal abnormalities. Both missense and protein truncating mutations in *NIPBL*, the human homolog of the *Drosophila Nipped-B* gene, have recently been reported to cause CdLS. The function of *NIPBL* in mammals is unknown. The *Drosophila* Nipped-B protein facilitates long-range enhancer-promoter interactions and plays a role in Notch signaling and other developmental pathways as well as being involved in mitotic sister chromatid cohesion. We report the spectrum and distribution of *NIPBL* mutations in a large, well-characterized cohort of individuals with CdLS. Mutations were found in 56 of 120 (47%) unrelated individuals with sporadic or familial CdLS. Statistically significant phenotypic differences between mutation-positive and mutation-negative individuals were identified. Analysis also suggested a trend towards a milder phenotype in individuals with missense mutations as compared to other types of mutations.

The phenotypic spectrum of exon skipping mutations in the COL1A2 gene. *F.L. Malfait, S. Symoens, P. Coucke, A. De Paepe.* Center for Medical Genetics , Ghent University Hospital, De Pintelaan 185, B-9000 Ghent, Belgium.

Mutations in the genes encoding collagen type I, COL1A1 and COL1A2, are commonly found in Osteogenesis Imperfecta (OI), but they occasionally result in an Ehlers-Danlos Syndrome (EDS). In EDS arthrochalasis type (EDS VIIA/B), skipping of exon 6 of COL1A1 or COL1A2 leads to loss of the cleavage site for procollagen I N-proteinase. Skipping of adjacent exons in COL1A2 have rarely been reported to result in an OI/EDS overlap phenotype. We present 2 new patients and compare clinical, electron-microscopic, biochemical and molecular findings. Patient 1 was diagnosed with EDS VIIA/B because of bilateral dislocation of the hip, hypotonia, joint hyperlaxity and recurrent luxations. She has a history of several fractures, but normal bone density. SDS-PAGE of procollagens and pepsin-digested collagens secreted in the medium and retained in the cell layer showed accumulation of pN1(I) and pN2(I) on the procollagen level, as well as presence of pN2(I) in the medium and the cell layer. Pulse chase experiments showed inefficient conversion of procollagen to collagen. Electron microscopy (EM) showed that the dermal collagen fibrils are round with mild surface irregularities in transverse sections. Molecular screening of COL1A2 revealed a heterozygous deletion of exon 6. Patient 2, a 36-year-old female, presents a phenotype overlapping OI and EDS with hyperextensible skin, moderate joint hypermobility, repetitive luxations, short stature, blue sclerae, osteoporosis and recurrent fractures. SDS-PAGE revealed no abnormalities, but pulse chase experiments showed inefficient conversion of procollagen to collagen. EM of dermal collagen fibrils is being performed. Molecular screening of COL1A2 revealed a heterozygous deletion of exon 7. Whereas skipping of COL1A2-exon 6 results in a characteristic EDS VIIB phenotype, due to loss of the procollagen I N proteinase cleavage site, skipping of adjacent exons, at the start of the triple helix, results in an OI/EDS overlap phenotype. The ligamentous laxity can probably be explained by incomplete processing of the N-propeptide, whereas the bone fragility is probably related to the structural alteration of the 2(I)-triple helix.

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Mendelian disorders: connecting genotype to phenotype in the Mouse Genome Informatics (MGI) system. *L.J. Maltais, C.M. Lutz, C.W. Goldsmith, C. Smith, D.L. Burkart, L.L. Washburn, P. Vanden Borre, H. Dene, M. Lennon-Pierce, I. Lu, J.T. Eppig, MGI Staff.* Bioinformatics, The Jackson Laboratory, Bar Harbor, ME.

Understanding the genetic contribution to disease is integral to effective treatment and prevention strategies. However, navigating and integrating the vast array of genetic and phenotypic information available is, like disease itself, complex. The Mouse Genome Informatics Database (www.informatics.jax.org/) provides integrated access to the genetics, genomics, and biology of the laboratory mouse, including phenotypic information on the many mouse models used for the study of human disease. MGI contains phenotypic information on approximately 10,000 spontaneous, induced, and genetically engineered mutant alleles representing nearly 4400 Mendelian genes. To better capture genotype to phenotype relationships, the Mammalian Phenotype Ontology (MPO), a structured vocabulary for describing phenotypes, has been developed. MPO contains over 3200 terms and facilitates consistent annotation and comparative analysis of phenotypic data. In addition to data on single gene mutations, MGI will utilize the MPO for integrating phenotypic descriptions of QTLs, complex traits, and strains (whole animal genotypes). To further enhance the genotype to phenotype to disease link, MGI provides associations between mouse phenotypes and Online Mendelian Inheritance in Man (OMIM), thus allowing comparisons with functionally or phenotypically similar human diseases and syndromes. Contextual information relating genotypes, expression, gene function, and comparative mapping augment the understanding of phenotypes within MGI. Phenotype representation integrated with other biological data will contribute to model building, the delineation and understanding of pathways, and the determination of the underlying mechanisms of genetic disease. Supported by NIH grant HG00330.

MOLECULAR STUDY OF OSTEOGENESIS IMPERFECTA IN BRAZILIAN PATIENTS. *F. Reis¹, C. Oliveira¹, F. Alexandrino¹, D. Cavalcanti², E. Sartorato¹*. 1) Lab de Genética Humana/CBMEG, UNICAMP, Campinas, São Paulo, Brasil; 2) Depto. Genética Médica/FCM - UNICAMP, Campinas - São Paulo, Brasil.

Osteogenesis imperfecta (OI) is a genetic disorder of increased bone fragility and low bone mass. Severity varies widely, ranging from intrauterine fractures and perinatal lethality to very mild forms without fractures. Most patients with a clinical diagnosis of OI have a mutation in one of the two genes that encode the α chains of collagen type I. The mild forms are usually caused by mutations which inactivate one allele of COL1A1 gene and result in a reduced amount of normal type I collagen, while the severe and lethal forms result from dominant negative mutations in COL1A1 or COL1A2 genes which produce structural defects in the collagen molecule. The aim of the present study was to identify OI-causing mutations in OI patients/families using direct sequencing of PCR products amplified from COL1A1 gene. This gene, located on chromosome 17, encodes the pro α 1 chain of type I procollagen and is composed of 52 exons scattered over 18 Kb of chromosomal material. Detection of mutations in type I collagen genes is complicated and difficult because of their large size and highly repetitive GC-rich coding regions. Here we report six mutations in COL1A1 gene in six unrelated Brazilian patients with OI types I-IV. Of them, four mutations appeared to be novel (c.1885delG, Pro239Arg, Gly592Ser and Gly649Asp) and the other two (Arg237Stop and Gly382Ser) were already described. The variety of mutations described in the COL1A1 gene giving rise to an OI phenotype is in accordance with the clinical heterogeneity of the disease.

FOXC2 Mutations are not associated with the Meige Disease (Lymphedema without Distichiasis). *T. Rezaie¹, R. Ghoroghchian¹, R. Bell², S. Jeffery², G. Brice², K. Burnand², J. Beninson², P. Mortimer², A. Child², M. Sarfarazi¹.* 1) University of Connecticut Health Center, Farmington, CT; 2) St Georges Hospital Medical School, London, UK.

Lymphedema-Distichiasis is an autosomal dominant disorder with clinical presentation of limbs edema and double row of eyelashes. This condition maps to 16q24.1 and mutations in FOXC2 gene have been reported in families with this disorder. We screened 16 Lymphedema families with a total of 81 living affected and additional 13 sporadic cases for mutations in FOXC2. The single coding exon of FOXC2 gene was PCR amplified and sequenced in 3 overlapping fragments. A 22-bp deletion was identified only in a single 3-generation family consisting of 8 affected members with Lymphedema-Distichiasis and 3 normal subjects. This deletion covers nucleotides at position 563-584 (ATG considered as base 1 in Y08223). Subcloning of PCR products into a TA cloning vector from both mutant and wild type alleles verified the nature of this deletion. This deletion located 17 amino acids after the Forkhead domain (nucleotides 211-510) and leads to truncation of the mature protein by 38%. This truncation eliminates a region at the carboxyterminal of FOXC2 Forkhead domain that is essential for interaction of FOXC2 with the transcriptional complex. This novel mutation segregates with the disease phenotype in all of the 8 affected but not with the 3 normal individuals of this family. Furthermore, this deletion was absent in a total of 202 normal control chromosomes. Therefore, co-segregation of this 22-bp deletion with the Lymphedema-Distichiasis phenotype in this family, its absence in unaffected individuals, and its truncating effect on the normal FOXC2 protein support the causative nature of this mutation. All the other 28 probands with Lymphedema but without Distichiasis (15 familial and 13 sporadic cases) did not show any mutations in the FOXC2 gene. Therefore, our study clearly shows that mutations in FOXC2 are limited to the Lymphedema families with Distichiasis but not with Meige Disease (Lymphedema without Distichiasis). Supported By: NIH-HL66150.

SMN2 copy number predicts acute or chronic SMA but does not account for intrafamilial variability in siblings.

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Spinal muscular atrophy (SMA) is an autosomal recessive disorder that affects motor neurons. It is caused by mutations in the survival motor neuron gene 1 (SMN1). The SMN2 gene, which is the highly homologous SMN1 copy that is present in all the patients, can be regarded as a naturally occurring hypofunctional allele of SMN1 and is unable to prevent the disease. We applied a quantitative SMN dosage method to assess the influence of the SMN2 copy number in patients with different SMA types (n=96) and in siblings of SMA patients with homozygous deletions or mutations in the SMN1 gene (n=6 pairs), whose disease outcome was notably different. Subsequent analysis and comparison were performed in these siblings to detect large-scale deletions or rearrangements in the SMA locus. Our results confirmed that the SMN2 copy number plays a key role in predicting acute or chronic SMA. However, siblings with discordant SMA phenotypes showed an identical SMN2 copy number and identical markers, indicating that the genetic background around the SMA locus is insufficient to account for the intrafamilial variability. Given that SMN2 is regarded as a target for potential pharmacological therapies in SMA, the identification of genetic factors other than the SMN genes is necessary to better understand the pathogenesis of the disease in order to implement additional therapeutic approaches. Supported by FIS 02-1275 and Red INERGEN C03-05.

Genotype-phenotype correlation in a large cohort of IP patients and identification of novel NEMO mutations interfering with NF- κ B activation. *M.V. Ursini¹, F. Fusco¹, G. Fimiani¹, A. Di Pardo¹, V. Mercadante², M.G. Miano¹, M. D'Urso¹.* 1) Institute of Genetics and Biophysics, Adriano Buzzati Traverso, CNR, Naples, Italy; 2) BioGeM, Ariano Irpino, Italy.

Incontinentia Pigmenti (IP) is an X-linked genodermatosis, lethal for males and presents in females with abnormal skin pigmentation and high variable clinical signs. NEMO gene, responsible for IP, encodes the regulatory subunit of the IKK complex for NF- κ B activation. We analyzed NEMO in 122 IP patients from EC and identified mutations in 83 (36 familiar and 47 sporadic cases). However, NEMO mutations isolated in this study account for the majority but not all clinical cases. The recurrent NEMO 4-10 deletion that is the major cause of the disease was present in 73 females. In addition 10 point alterations were identified. A phenotype scoring system was used for correlation between the mutation type and the clinical IP presentation. To assess genotype-phenotype correlation in IP patients we listed features that are typical for IP but not consistent in all patients and could therefore reflect the severity of the syndrome. For calculating the score we included only defects relative to nervous system, eyes, teeth, hair and nail, that are most frequently, but not always, observed in IP patients. If a genotype-phenotype correlation does exist, females who carry loss-of-function mutations would be expected to have the most severe phenotype. In our study, however, this is not the case: 50 patients with the same exon 4-10 NEMO deletion have severity score ranging from 1 to 8. In addition, we found that mutations preserving some activity (i.e. same point mutations) show an atypical phenotype characterized by involvement of much more tissues than classical IP phenotype. Thus, the detected mutation spectrum do not allow genotypephenotype correlation, leading to the hypothesis that other factors may modulate the IP pathogenesis. Such a factor is likely to be X-inactivation. Indeed, 64% of our patients have extremely skewed X-inactivation pattern (80:20). Overall IP pathogenesis thus depends on a combination of X-inactivation and protein domain that recruit upstream factors and activate NF- κ B.

Analysis of the behavioral phenotypic consequences of gene dosage imbalance utilizing the mouse models for $\text{del}(17)(\text{p}11.2\text{p}11.2)$ and $\text{dup}(17)(\text{p}11.2\text{p}11.2)$ contiguous gene syndromes. *K. Walz, R. Paylor, J.R. Lupski.*
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Contiguous gene syndromes (CGS) refer to a group of disorders associated with chromosomal rearrangements in which the phenotype is thought to result from altered copy number of physically linked dosage sensitive genes. Smith-Magenis syndrome (SMS) is a CGS associated with a deletion within band p11.2 of chromosome 17. Patients harboring the predicted reciprocal duplication product [$\text{dup}(17)(\text{p}11.2\text{p}11.2)$] have also been described. We have previously described the construction of mouse models for these two independent CGS. Taking advantage of the availability of animals harboring different gene dosage in this specific interval (0, 1, 2, 3 and 4n) we wanted to study the phenotypic consequences of this imbalance. We analyzed here the viability of the different groups of animals. Pathological and histological studies were performed for most of these genotypes. In addition a battery of tests was utilized to study the behavior. While homozygous deletion animals are lethal, homozygous duplication animals present a severe compromise in their viability, only a few survive the neonatal period. All the others genotypes are present at the normal mendelian ratios. Preliminary behavior analysis indicate that the homozygous duplication ($\text{Dp}(11)17/\text{Dp}(11)/17$) animals have a more profound phenotype when compared with the heterozygous duplication ($\text{Dp}(11)17/+$) and heterozygous deletion ($\text{Df}(11)17/+$) animals, and also presented a learning and memory deficiency in the pavlovian conditioned fear, not only to the context (as the duplication heterozygous), but also to the acoustic cue. Our murine models clearly demonstrate that there are specific genes in this interval that are dosage sensitive genes, and that behavior is influenced by having either too few (0-1n) or too much (3-4n) of at least one of these genes products.

CFTR Gene Haplotype Determines the Phenotype Associated with the 5T Variant. *B. Anderson, W. Sun, J. Redman, A. Buller, M. McGinniss, F. Quan, C. Strom.* Dept Molecular Genetics, Quest Diagnostics Nichols Institute, San Juan Capistrano, CA.

Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene are responsible for the disease in almost all CF patients. Mild CFTR mutations and variants have been associated with atypical clinical presentations including congenital bilateral absence of the vas deferens (CBAVD), chronic pancreatitis, and chronic rhinosinusitis. A tract of 5 thymidines in intron 8 of the CFTR gene, the 5T variant, is a common CF variant, with an allelic frequency of 0.04 in our test population. It disrupts processing of CFTR mRNA and thus results in less gene product being made. When found on the opposite chromosome from a severe CF mutation, the 5T variant has been associated with a normal phenotype or with male infertility stemming from CBAVD or atypical CF. To better understand the molecular basis for these different clinical phenotypes, we analyzed the polymorphic TG dinucleotide repeat adjacent to the 5T variant in intron 8 as well as codon 470 in exon 10. All patients selected for this study were positive for both the 5T variant and the major CF mutation, Delta F508, as our study showed that the CFTR gene containing the Delta F508 mutation appeared to have the 10TG-9T-470M haplotype. Out of the 65 samples analyzed by direct sequence analysis, 39 (60%) were 11TG-5T-470M, while 24 (37%) were 12TG-5T-470V, and 2 (3%) were 13TG-5T-470M. Of the 44 cases for which we had clinical information, 33 (75%) were normal (23 with 11TG, 8 with 12TG and 2 with 13TG) and 11 (25%) had some atypical CFTR-related symptoms (7 with 12TG and 4 with 11TG). Thus, 7 of the 15 cases having a 12TG haplotype had an abnormal phenotype versus only 4 of the 27 cases having 11TG haplotype, suggesting that a 12TG-5T-470V haplotype is more likely to cause atypical CF than an 11TG-5T-470M haplotype. In view of these results (low penetrance and 12TG association with abnormal phenotype), haplotype determination may be useful for clinical interpretation of a 5T variant. Additional studies are needed to verify our results.

HAPLOTYPIC CLASSIFICATION OF THE DYSTROPHIC EPIDERMOLYSIS BULLOSA IN TUNISIAN FAMILIES. *H. OURAGINI*¹, *W. DAOUD*², *F. CHERIF*², *A. BEN OSMAN*², *K. DELLAGI*¹, *S. ABDELHAK*¹. 1) Laboratory of Immunology, Vaccinology and Molecular genetics, Institut Pasteur de Tunis, Tunis, Tunisia; 2) Departement of Dermatology, Hospital of La Rabta, Tunis, Tunisia.

Dystrophic Epidermolysis Bullosa (DEB) is a rare skin disorder that is characterized by tissue separation under the dermal-epidermal basement membrane zone, because of an alteration of the anchoring fibrils (AF). COL7A1 gene encodes for type VII collagen, the major component of the AF. Mutations within this gene have been shown to be responsible for DEB. The COL7A1 gene is composed of 118 exons and localised at 3p21.3. DEB is transmitted either in a dominant or a recessive mode. More than 200 mutations have been identified. These mutations consist of nonsense mutation, small insertion or deletion. Our aim is to classify the DEB Tunisian patients on the basis of their haplotype. Sixteen families have been typed with three markers overlapping the COL7A1 gene region. At least two haplotypes (222-252-276 and 226-246-290) have been identified. In a family, a pseudo-dominant mode of inheritance have been suspected. A neomutation in one of the tested markers, has been also identified within this family. This preliminary study is essential for mutation screening.

Exclusion of candidate genes in a french-canadian cohort with a milder form of UCMD. *M. Tetreault*^{1,2}, *A. Duquette*¹, *I. Gosselin*¹, *A. Klein*^{1,2}, *M. Vanasse*³, *Y. Robitaille*³, *G. Karpati*⁴, *B. Brais*^{1,2}. 1) Neurogenetic, CHUM-Hopital Notre-Dame, Montral, Quebec, Canada; 2) Molecular Biology Departement, University of Montreal, Quebec, Canada; 3) Hopital Ste-Justine, Montreal, Quebec, Canada; 4) Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada.

Ullrich congenital muscular dystrophy (UCMD) is a heterogeneous autosomal recessive dystrophy associated with proximal contractures and distal hyperlaxity. UCMD has been associated with complete or partial loss of collagen VI due to mutations in genes coding for its three subunits. This rare hereditary disorder account for 60% of all CMDs in Quebec. Most UCMD French-Canadian cases have a mild phenotype. Objective: Establish if the higher prevalence of UCMD in the French-Canadian population is due to mutations in one of the subunits of collagen VI and if not screen genes loci coding for proteins interacting with collagen VI. Methods: We have recruited 14 affected patients from 11 UCMD families in Quebec for the clinical and genetic characterization of UCMD in French Canadians. DNA and muscle biopsies from affected patients and DNA samples from relatives were obtained following informed consent. We have completed haplotype analyses in all families with polymorphic markers at the *COL6A1-A2* and *COL6A3* loci. Linkage analysis were also performed at the *HSPG2*, *COL4A3-COL4A4*, *DAG1*, *ITGA2*, *DCN*, *COL2A1*, *COL4A1-COL4A2* loci. Using RT-PCR, the mRNA for *COL6A3* was completely sequenced in three cases. Immunofluorescent microscopy was performed on available muscle and culture fibroblasts using the anti-collagen VI antibody. Results: Haplotype analyses excluded that a founder mutation in the *COL6A1* and *COL6A2* genes is responsible for UCMD in our cohort. Similar analysis at the *COL6A3* locus was not conclusive. The sequencing of *COL6A3* gene by RT-PCR in three cases did not uncover any mutations. Immunofluorescence microscopy demonstrated the presence of collagen VI in the four studied cases. The haplotype analysis performed at the other loci excluded them as candidate genes in our cohort. Together our results indicates that the milder form of UCMD in French Canadians is a distinct disease genetically.

A Novel Mutation In The Uroporphyrinogen III Synthase (UROS) Gene Causes Congenital Erythropoietic Porphyria In A Pakistani Family. *A. Abid, M. Ismail, S. Khaliq, S.Q. Mehdi.* Biomedical and Genetic Engineering Laboratories, Islamabad, Pakistan.

Porphyrias are inherited disorders of heme biosynthesis. Congenital erythropoietic porphyria (CEP) or Gunther disease is a rare autosomal recessive disease caused due the deficient activity of uroporphyrinogen III synthase (URO3S) enzyme. CEP is typically expressed at birth or in early childhood. However, a few cases of adult-onset CEP have also been reported. It is characterized by the accumulation of porphyrins in erythrocytes and bone marrow and excreted in the urine and feces at later stages. In this study a consanguineous Pakistani family, with 4 affected members, in which CEP phenotype segregated as an autosomal recessive trait were investigated. The affected members show signs of hemolytic anemia and scarring of the skin. The nails, hairs and teeth of the patients were pink or dark brown due to the accumulation of large amount of porphyrins. High levels of porphyrins were also excreted in the urine and feces. Progressive degeneration of digits, ears, nose and vision was also observed in one severely affected patient aged 28y. Linkage analysis of this family mapped the disease locus on chromosome 10q25.2-q26.3, a region harboring the Uroporphyrinogen III synthase gene (UROS) gene. Mutation screening of the UROS gene revealed a novel T to C homozygous substitution in exon 10 in all the patients of 1CEP family. The parents were heterozygous (carriers) for the mutation. This mutation alters the codon 237 for leucine to proline (CTT to CCT) that abolishes the N-myristoylation site in the mutated protein product resulting in the dysfunction of the biochemical pathway. These results indicate that this novel mutation may be the cause of CEP in this family.

Towards the identification of the gene(s) causing Dup(17)(p11.2p11.2) by BAC transgenesis. *P. Fonseca, K. Walz, R. Paylor, J. Lupski.* Dept Human Molecular Genetics, Baylor Col Medicine, Houston, TX.

Smith-Magenis Syndrome and the reciprocal Dup(17)(p11.2p11.2) Syndrome are independent genomic disorders associated with a deletion/duplication within band p11.2 of chromosome 17. Engineered mouse models for both syndromes suggest the presence of dosage-sensitive gene(s) in the region. Although mutations in *RAI1* were associated with Smith-Magenis Syndrome, it is still an open question whether the same gene is responsible for the clinical features observed in patients harboring a duplication of this chromosomal region. We generated three different lines of transgenic mice containing 3 candidate dosage-sensitive genes to generate an overexpression model that recapitulates the phenotype of the Dup(17)(p11.2p11.2) Syndrome mouse model, to rescue the phenotype of the SMS mouse model and to identify dosage sensitive genes in the SMS critical region. These animals were subjected to several tests that evaluate different domains of CNS that were abnormal for mice harboring the duplication. Preliminary data for *DEXRAS/RASD1* transgenic mice showed no significant differences between transgenic animals (N=8) and wild-type littermates (N=3) in the open-field exploratory (average total distance: 290994.20 cm (wt), 2437170.0 (transgenic), $p=0.14$; average movement time: 32224.5 sec (wt), 27719.0 (transgenic), $p=0.22$) and light-dark tests (average number of transitions: 268.8 (wt), 203.1 (transgenic), $p=0.4$). There were also no significant differences in any parameter of the prepulse inhibition of the acoustic startle response or the conditioned fear tests (percentage freezing to the context: 719.5 wt, 577.1 transgenic, p value=0.31; percentage freezing to the sound: 723.2 wt, 588.3 transgenic, p value=0.6). These data suggest that *RASD1* is not the dosage sensitive gene causing the behavioral abnormalities seen in the Dp(17)(11) mouse. *DRG2* transgenic mice do not present any obvious abnormality and behavioral tests are in progress. Transgenic mice carrying the human *RAI1* gene, as well as the murine cDNA, are currently being analyzed for the behavioral features and weight phenotype observed in the Dup(17)(p11.2p11.2) mouse model.

RAI1DEXRAS/RASD1RASD1DRG2.

Brachydactyly Type A1 (BDA1): Identification and Characterization of the Genes Involved. *A. Grimsey*^{1,3}, *E. McCready*^{1,3}, *C. Armour*¹, *S. Nikkel*², *A. Hunter*², *D. Bulman*^{1,3,4}. 1) Ottawa Health Research Institute; 2) Division of Genetics, CHEO; 3) Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa and Neuromuscular Research Group; 4) Department of Medicine, Division of Neurology, University of Ottawa, Ottawa.

Brachydactyly Type A1 (BDA1) belongs to a group of rare, congenital disorders whereby normal bone development and patterning is adversely affected, leading to shortened and malformed digits. BDA1 has the unique feature of being the first human trait described in terms of autosomal dominant Mendelian inheritance. A missense mutation within the Indian hedgehog (IHH) gene at codons 95, 100 or 131 are known to cause this disorder. IHH is essential for bone development as it regulates proliferation and differentiation of chondrocytes. We recently identified linkage of BDA1 to a second locus on chromosome 5p13.3 in a large Canadian kindred indicating BDA1 is a genetically heterogeneous disease. The critical region on chromosome 5p has been refined to 7cM (3.5-4Mb) by genotype and haplotype analysis of family members, and the detection of a common haplotype that cosegregates with BDA1. Our main objective is to identify the BDA1 gene on chromosome 5 and characterize its involvement in skeletal development. Future directions include acquiring other BDA1 family members and new BDA1 families to aid in refining the position of the BDA1 locus on chromosome 5p, eventually leading to its identification and characterization.

New loci for Fraser Syndrome and mouse blebbing mutants. *S. Jadeja*¹, *I. Smyth*¹, *J. Pitera*^{1,2}, *M. van Haelst*¹, *C. Roberts*¹, *S. Darling*¹, *A.S. Woolf*², *P.J. Scambler*¹. 1) Molecular Medicine Unit, Institute of Child Health, London, United Kingdom; 2) Nephrourology Unit, Institute of Child Health, London, United Kingdom.

Fraser syndrome is a multisystem malformation usually comprising cryptophthalmos, syndactyly and renal defects; other defects occur more rarely. The condition is genetically heterogeneous with the first disease locus (*FRAS1*) having been identified. The mouse blebbed mutants are a group of five mouse strains with cryptophthalmos, syndactyly and renal defects that provide a model for Fraser syndrome. One of these mutants blebbed (*bl*) has been shown to carry a mutation in *Fras1*. The FRAS1 protein shares homology with the sea urchin extracellular matrix (ECM) blastocoelar protein ECM3. The domain structure of these proteins suggests a structural role in the extracellular matrix as well as in cell signalling. The blebbed mutants have a separation of the dermis from the epidermis, below the basement membrane.

Linkage analysis and homology searching has identified a second disease locus *FREM2* (FRAS1-like Extracellular Matrix gene/ protein), in which a missense mutation (E2041K) has been found. The murine orthologue of *FREM2* is closely linked to the *my* (myelencephalic) blebs locus. Expression analysis of *my* has revealed epithelial and renal localization. In the *bl* mutants, collagen IV and VI expression is lost, however; collagen IV is present in *my* mice. Mutation screening of *Fras2* has yet to identify a mutation, although a gene trap mutant is available and tests for allelism are underway.

To investigate the affect the different blebbed strains would have on each other, compound mutants were created by crossing the *bl* and *my* strains. Double homozygote mice (*blbl/mymy*) are viable but present with a severe renal, limb and eye phenotype.

These mouse models for Fraser syndrome should provide important insights into the development of epithelial structures, as well as eye and kidney development.

X-linked cone-rod dystrophy (COD4) in a Finnish family is caused by mutation in *CACNA1F*. R. Jalkanen¹, M. Mäntyjärvi², R. Tobias³, T. Alitalo¹, N.T. Bech-Hansen³. 1) Biomedicum Helsinki, Department of Obstetrics and Gynecology, Helsinki University Central Hospital, Helsinki, Finland; 2) Department of Ophthalmology, University of Kuopio, Kuopio, Finland; 3) Department of Medical Genetics, University of Calgary, Calgary, Alberta, Canada.

X-linked cone-rod dystrophy (COD) is a progressive retinal disease primarily affecting the cone photoreceptors. Affected males show reduced visual acuity, moderate to high myopia, photophobia, defects in color vision, central scotomas, and affected cone or cone-rod ERG responses. The disease is genetically heterogeneous and at least three genes are known to be behind the disorder. COD1 is caused by mutations in the *RPGR* gene (Xp21.1). Mutations of the *RPGR* gene are also responsible for RP3 type retinitis pigmentosa. COD2 has been localized to the long arm of the X-chromosome (Xq27.2-28). We have recently localized the third X-linked COD locus, *COD4*, on Xp11.4-q13.1 (*J Med Genet* 40:418-423).

The aim of our study was to identify the disease gene behind the COD4 phenotype. We sequenced a number of genes in the candidate region to find a disease causing mutation. Using direct PCR sequencing we screened all 48 exons of the *CACNA1F* gene (Xp11.23). A novel mutation in the splice acceptor site of intron 28 co-segregated with the disease in the Finnish X-linked COD family, and was not observed in 100 control chromosomes. This sequence change may lead to skipping of exon 29 or an alternative acceptor site upstream of exon 29.

Mutations of *CACNA1F* are known to cause two other retinal diseases: incomplete form of X-linked congenital stationary night blindness (CSNB2), including an Åland Island eye disease -like phenotype, and retinal and optic disc atrophy. Our results indicate that yet another phenotype, COD4, is caused by a mutation in the *CACNA1F* gene.

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Polymorphisms of Wnt1 inducible signalling pathway protein 3 (WISP3) and susceptibility to Juvenile Idiopathic Arthritis. *R.M. Lamb¹, W. Thomson¹, E. Ogilvie³, R. Donn^{1,2}, The British Society of Paediatric and Adolescent Rheumatology.* 1) ARC/EU, University of Manchester, Manchester, England, UK; 2) Centre for Molecular Medicine, University of Manchester, UK; 3) UCL, London, UK.

Background: Juvenile Idiopathic Arthritis (JIA) is the most common chronic rheumatic disease of childhood. Progressive Pseudorheumatoid Dysplasia (PPD) is a rare skeletal disorder, which clinically mimics JIA. Mutations of the WISP3 gene are causal of PPD. WISP3 SNPs are therefore important candidates for investigation in JIA. **Aims:** To identify WISP3 SNPs and determine their role in JIA. **Methods:** Exonic mutation screening by dHPLC was performed in 86 UK Caucasian JIA cases and 20 controls. Genotyping for the identified SNPs, together with publicly available WISP3 promoter and 3UTR SNPs, was by allelic discrimination. In total seven SNPs were studied in 612 unrelated controls and, initially, 372 UK Caucasian JIA cases (Cohort 1). For replication a separate cohort of 372 UK Caucasian JIA cases (Cohort 2) were used. Comparison of genotype and allele frequencies was by the χ^2 test. Haplotype frequencies, inferred by the EM algorithm, were compared using HelixTree (GoldenHelix Inc). **Results:** Two WISP3 SNPs were associated in both JIA cohorts. Specifically, homozygosity (TT) of rs2280153 (Cohort 1 OR 1.92 95%CI 1.143.24, p=0.008; Cohort 2 OR 1.7 95%CI 12.9, p=0.036) and possession of the mutant allele rs971531A (Cohort 1 OR 1.2 95%CI 11.5, p=0.05; Cohort 2 OR 1.2 95%CI 11.5, p=0.04) is associated with increased risk of JIA susceptibility. Comparison between JIA subgroups showed no significant differences. Although strong LD exists between the associated SNPs in both JIA cases and controls ($r^2=0.9$, $D=0.9$) no significant haplotypic association of the WISP3 SNPs and JIA susceptibility was found. **Discussion:** Replication of association of the two SNPs, rs2280153 within intron 1 of the WISP3 gene and the 3UTR SNP rs971531 with JIA susceptibility was seen. Further analysis is underway in TDT families to identify linkage and association. Functional studies of linked and associated SNPs will then follow.

ANALYSIS OF INTRON 1 AND 22 INVERSIONS IN FACTOR VIII GENE IN SEVERE HEMOPHILIA A MEXICAN PATIENTS. *J. Mantilla¹, A.R. Jaloma-Cruz¹, C.P. Beltran-Miranda¹, A. Esparza², B. Lopez-Guido², L. Aguilar-Lopez².* 1) Division de Genetica, Centro de Investigacion Biomedica de Occidente. IMSS. Guadalajara, Mexico; 2) Division de Hematologia. Centro Medico Nacional de Occidente, IMSS. Guadalajara, Mexico.

Hemophilia A (HA) is caused by mutations in factor VIII gene (FVIII), it is the most common sex-linked inherited bleeding disorder. The severe disease is caused by FVIII gene intron 22 inversion (inv22) in approximately 50% of patients and by intron 1 inversion (inv1) in 5%. Both inversions result from an intra-chromosomal recombination between homologous regions, at intron 1 or 22, and their extragenic copies telomerically located from FVIII gene. The goal of the present study is to analyze the presence of large structural changes in the FVIII gene in Mexican severe HA patients. **METHODS:** We studied 102 severe HA patients from 69 families. We based our protocol on the modification to the subcycling-long distance PCR (by individual reactions) for detection of inv22 and rapid PCR for detection of inv1, both procedures for the simultaneous detection of rearrangements in patients and carriers. **RESULTS:** Until the moment we found the inv22 in 47% (14/30) of severe HA patients. After the screening of inv1 in all our severe HA population we identified only wild type individuals. **DISCUSSION:** We support the observation that intron 22 represents a hot spot of mutations in FVIII gene in severe HA patients of Mexico in similar way to other populations. In contrast, in Mexico inv1 frequency it is significant lower than in diverse European populations. **CONCLUSIONS:** The frequency of inv22 is similar to other populations, while intron 1 inversion seems to be not common in Mexicans.

Toward the identification of a new gene responsible of anhidrotic ectodermal dysplasia. *A. Munnich, A. Morlon, L. Faivre, S. Hadj-Rabia, A. Smahi.* Dept Ped Genetics, Inserm U393, Hosp Enfants Malades, Paris, paris, France.

Ectodermal dysplasia (ED) is a clinically heterogeneous condition characterized by the abnormal development of ectoderm-derived structures including teeth, hair, nails and eccrine sweat glands. The anhidrotic form (EDA) is characterized by the association of sparse hair (atrachosis or hypotrichosis), abnormal or missing teeth (anodontia or hypodontia) and the inability to sweat (anhidrosis) which is responsible for life-threatening brain-damaging episodes due to hyperthermia. The most common type of EDA (MIM 305100) is a X-linked recessive disorder caused by mutations in the ectodysplasin gene (ED1), a member of TNF (Tumor Necrosis Factor) cytokine superfamily. Autosomal dominant and recessive forms of EDA have been also reported (MIM 224900) and are allelic at the ED3 locus that encodes EDAR a protein belonging to the TNF receptor superfamily. Mutations in the EDARADD gene are also responsible of autosomal recessive EDA. During skin appendages morphogenesis, EDAR is activated by Ectodysplasin and uses EDARADD as an adapter to build a signal-transducing complex leading to NF- κ B activation. We present a three-generation family with an autosomal dominant EDA. Among the 55 members of this family, 17 individuals were affected and presented the classical phenotype of EDA with marked dental abnormalities. By linkage analysis, we excluded the ED3 (2q13) and EDARADD (1q42) loci. We also excluded XEDAR (Xq12), Troy (13q12.12) and TRAF6 (11p12) genes which are candidate genes for anhidrotic ectodermal dysplasia. We performed a genome wide search and are currently investigating the candidate regions.

Identification of the gene mutated in Amish Brittle Hair (Hair Brain) syndrome. *K. Nakabayashi¹, D. Amann², N. Avidan², Y. Ren¹, U. Kere³, S. Gentles¹, J. MacDonald¹, E.G. Puffenberger⁴, A. Martinez-Mir⁵, J. Salas⁵, A. Christiano⁵, C. Les⁶, E. Seboun⁷, J. Beckmann², C.E. Jackson⁸, S.W. Scherer¹.* 1) Dept. of Genet., The Hosp. for Sick Children, Toronto; 2) Dept. of Mol. Genet., Weizmann Inst. of Science, Rehovot, Israel; 3) Dept. of Dermatol., Univ. of Helsinki, Finland; 4) The Clinic for Special Children, Strasburg, PA; 5) Dept. of Dermatol., Columbia Univ., New York, NY; 6) Henry Ford Hosp., Detroit, MI; 7) Div. of Genet. and Microbiol., Univ. Pierre et Marie Curie, Paris, France; 8) Dept. of Med., Scott & White Memorial Hosp., Temple, TX.

Amish Brittle Hair syndrome (OMIM:234050) is an autosomal recessive disorder characterized by short stature, intellectual impairment, brittle hair, and decreased male fertility. The patients hair shows low sulfur content, an irregular and grooved surface on scanning electron microscopy and distinct changes on polarization microscopy. Sabinas syndrome, trichorrhexis nodosa with mental defect, and Pollitt syndrome have been suggested to be the same disease with Brittle Hair syndrome. Homozygosity mapping on a subset of affected members from a large Amish consanguineous kindred identified a 2 Mb candidate locus on chromosome 7p. Mutation screening of 7 candidate genes within the interval identified a homozygous sequence variation that causes an amino acid change (methionine to valine) in one of the genes. Further mutational analysis revealed that all 13 affected members are homozygous for the sequence variation, and 28 unaffected members are either heterozygous for the variation or homozygous for the normal allele (150 controls including 48 Amish individuals were found to be homozygous for the normal allele). These data strongly suggest that this sequence variation is causal for the disease phenotypes in the kindred. Involvement of this gene with Sabinas syndrome has been excluded by the lack of linkage to the 7p locus. The gene is predicted to encode a 180 amino-acid (aa) protein, which does not contain any known motifs. The mutated aa residue is located in a fifteen aa sequence that is highly conserved from fly to mammals. Characterizations of gene expression patterns and protein functions are underway.

Characterisation of a de novo translocation in a patient with isolated respiratory complex I deficiency. *L. Parry¹, E. Haquet¹, D.R. Thorburn^{1,2,3}, H. Dahl^{1,2}.* 1) Murdoch Children Research Institute, Royal Childrens Hospital, Flemington Rd, Parkville, Melbourne, VIC 3052, Australia; 2) Genetic Health Services Victoria, Royal Childrens Hospital, Parkville, Melbourne, VIC 3052, Australia; 3) Department of Paediatrics, University of Melbourne, Melbourne, Australia.

With a minimum birth prevalence of 1/5,000, primary respiratory chain disorders are one of the most common forms of neuromuscular disease. Respiratory complex I (CoI) deficiency, the most prevalent respiratory chain disorder, is a genetically heterogeneous condition. Although mutations have been identified in eight nuclear subunits and seven mitochondrial DNA genes, the etiology of most CoI cases remain unknown. In the majority of cases the families are not suitable for linkage analysis. Furthermore, little is known about the factors needed for assembling and modifying the CoI complex. As a consequence it has been difficult to identify novel genes responsible for CoI deficiency and alternative approaches are needed. We have identified a unique patient with a de novo chromosome 11:18 translocation [t(11:18)(p12;q21.3)]. FISH analysis identified BACs, which spanned the breakpoints on the derivative chromosomes. Currently for chromosome 11 southern analysis has reduced the 199kb region identified by BAC RP11-768P16 to ~70kb. For chromosome 18 the 146kb region identified by BAC R145-D03A has been reduced to 35kb, with a ~0.5Mb inversion identified adjacent to this. Neither of the regions disrupted by the rearrangements disrupts any known genes. Analysis of ESTs within the region has identified a single EST which was expressed in control cDNA but absent in patient cDNA. Here we present data on the refinement of the complex genomic rearrangement, analysis of EST sequences expressed in the region and microarray analysis of global gene expression in patient cells.

Familial tumoral calcinosis is an O-glycosylation disorder due to loss of function mutations of GALNT3 and is genetically heterogeneous. G. Richard¹, O. Topaz^{2, 3}, R. Bergman^{2, 3}, D.L. Shurman¹, M. Indelman², R. Ratajczak¹, M. Mizrachi^{2, 3}, Z. Khamaysi², D. Behar³, D. Petronius², V. Friedman³, I. Zelikovic^{2, 3}, S. Raimer⁴, A. Metzker⁵, E. Sprecher^{2, 3}. 1) Thomas Jefferson University, Philadelphia, PA; 2) Rambam Medical Center, Haifa, Israel; 3) Technion-Israel Institute of Technology, Haifa, Israel; 4) University Texas Medical Branch, Galveston, TX; 5) Tel Aviv-Sourasky Medical Center, Israel.

Familial tumoral calcinosis (FTC) is an autosomal recessive metabolic disorder with normal or elevated serum phosphate levels characterized by massive, painful calcium deposits in the skin and subcutaneous tissues resulting in profound disability and mutilation. We mapped hyperphosphatemic FTC in 2 large kindreds of different origins to a 3 Mb region in 2q24-q31 while 4 families with normophosphatemic FTC showed no linkage, suggesting that normo- and hyperphosphatemic FTC are not allelic. The 2q region harbors the *GALNT3* gene encoding a member of the GalNAc-transferases family (ppGalNAc-T3) responsible for initiating mucin-type O-glycosylation. RT-PCR analysis demonstrated expression of *GALNT3* in the skin, kidney, bone marrow, lung and other epithelial organs. DNA sequencing revealed 3 pathogenic *GALNT3* mutations, a nonsense mutation in exon1 and 2 distinct nucleotide transitions altering the donor splice site in intron 7, segregating in the hyperphosphatemic FTC families. The mutations in both families were shown to abolish full-length *GALNT3* transcripts and, in one family, resulted in low production of an aberrant splice variant lacking 44 residues due to skipping of exon 7. Nevertheless, immunostaining of ppGalNAc-T3 in affected epidermis was completely absent. ELISA tests demonstrated that serum levels of FGF23, a key regulator of circulating phosphate levels, were significantly elevated in FTC patients, suggesting a compensatory upregulation of FGF23 or decreased FGF23 clearance. Our results demonstrate for the first time the pathological consequences of a genetic defect in a mucin-type O-glycosylation pathway and implicate ppGalNAc-T3 in the regulation of phosphate homeostasis and basic calcium crystal formation.

Recovery, additional characterization and further mapping of mouse coralliform cataract (Coc) locus. D.

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Cataracts, or lens opacities, are the leading cause of blindness worldwide. The coralliform cataract (Coc) is a murine autosomal dominant locus recovered in the offspring of a 5.1 + 5.1 Gy X-irradiated male. Both Coc/Coc and Coc/+ were shown to exhibit small spherical opacities in the central part of the lens. The Coc locus was mapped to 28 cM of chr. 16 in the region with synteny with HSA3p12-q21 and cryopreserved. In this study we recovered the Coc mice from Coc/+ sperm, further clinically described the affected mice and generated additional backcross progeny. Utilizing a standard IVF techniques 6 mice (5 males and 1 female) were generated. On a slit lamp examination two recovered males showed no signs of lens opacities. The remaining four littermates (three males and a female) all showed microphthalmia, squinting of the eyes, coralliform nuclear cataracts and were presumed to be Coc/+. The recovered Coc/+ mice were outcrossed to C57BL/6 and mice (C3H Coc/+ x C57BL/6) that showed cataracts (Coc/+) were then backcrossed to C57BL/6 to generate a backcross panel for further mapping. The cataract phenotype of affected backcross animals was similar to the parental phenotype. Further mapping of the Coc locus is currently in progress. In addition to generating a backcross panel we set up a cross of heterozygotes (Coc/+ x Coc/+). A litter of six mice was generated and at weaning no ocular abnormalities were detected in 4 littermates. The two remaining littermates appeared to be affected: one showed microphthalmia, squinting of the eyes and coralliform nuclear cataract similar to the parental phenotype; the other mouse had unopened sinking eyelids suggesting absence of both eyes. Both affected mice were smaller in size relative to the wild type littermates. Further matings have been set up to generate additional Coc animals for histological analysis.

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A new mutation of *EFHC1* associating with juvenile myoclonic epilepsy. T. Suzuki¹, A.V. Delgado-Escueta², K. Aguan¹, M.E. Alonso³, J. Shi¹, M.T. Medina⁴, T. Takeuchi¹, D. Bai², J.N. Bailey², A. Ochoa³, A. Jara-Prado³, A. Rasmussen³, F. Rubio-Donnadieu³, K. Yamakawa¹. 1) Lab. for Neurogenetics, RIKEN BSI, Saitama, Japan; 2) Epilepsy Genetics/Genomics Laboratories, Comprehensive Epilepsy Program, UCLA School of Medicine and VA GLAHS-West Los Angeles, Los Angeles, CA; 3) National Institute of Neurology and Neurosurgery, Mexico City, Mexico; 4) National Autonomous University, Tegucigalpa, Honduras.

Among idiopathic generalized epilepsies, juvenile myoclonic epilepsy (JME) is the most common, accounting for 10-20 % of all epilepsies. Previously we mapped one of the candidate regions on chromosome 6p12-p11 (*EJMI*), and narrowed it to the 3.5cM region (Bai *et al.* Am J Med Genet 113:268-274, 2002). Recently we reported JME mutations of a new gene *EFHC1* in the *EJMI* region and functional copromises of EFHC1 protein by the JME mutations (Suzuki *et al.*, 2004 in press). We then further recruited new JME families, performed mutation analyses on these families, and identified an additional novel missense mutation of *EFHC1* in the mutation hot spot region further suggesting the functional significance of the region. We also show the functional effects of EFHC1 with the mutation on apoptosis.

Canadian Cystic Fibrosis Modifier Gene Project. *J. Zielenski¹, A. Sandford², M. Corey¹, R. Dorfman¹, D. Markiewicz¹, X-W. Yuan¹, A. Master¹, I. Evtoushenko¹, G. Deng¹, M. Patel¹, I. Aznarez¹, M. Tan¹, F. Lin¹, P.D. Paré², L-C. Tsui^{3,1}, P. Durie¹, and Canadian CF Clinics.* 1) Hospital for Sick Children, Toronto, Canada; 2) iCAPTURE/UBC, Vancouver, Canada; 3) University of Hong Kong, Hong Kong.

Cystic fibrosis (CF) is a monogenic disease caused by mutations in the cystic fibrosis transmembrane regulator (CFTR) gene. The phenotypic variability of CF is influenced by CFTR mutations as well as variations in other genes (modifiers). Due to the complex nature of these influences, genetic analyses of large populations are recommended. To this end, the Canadian CF Modifier Gene Project has been launched, aiming at the mapping and isolation of genes influencing severity of disease in CF. The Project involves: (1) blood collection from Canadian CF families; (2) clinical data collection from CF patients; (3) re-structuring and updating Canadian CF Patient Data Registry; (4) CF modifier candidate gene analysis and (5) genome linkage analysis to detect CF modifier loci. To date, 30 Canadian CF Clinics have obtained consents from 1128 CF families (target: ~2300 families). Genomic DNA was extracted from 4858 blood samples collected from patients and parents. In addition, 923 lymphoblast cell lines have been established. Extensive cross-sectional and longitudinal clinical data is being collected from medical charts of CF patients. Approximately 80% of the data have been securely submitted to the database from charts of participating patients. Testing of genes and loci as potential CF modifiers is performed using two approaches: candidate gene analyses and genomic scans. Currently, 57 genes with relevance to CF have been selected as potential modifiers. Genotyping of 89 intragenic markers is in various stages of completion. Also, a chromosome 6qter locus is being tested in CF families as a putative modifier of pulmonary function. The progress in these areas of the project is presented in separate abstracts. In order to oversee, regulate and govern resources created in the current Project, the Canadian Consortium for CF Modifier Studies is being established (Supported by Genome Canada and Canadian CF Foundation).

Imprinting and acid sphingomyelinase deficient Niemann-Pick disease. C. Simonaro, J. Park, E. Schuchman. Dept Human Genetics, Mount Sinai Sch Medicine, New York, NY.

An inherited deficiency of acid sphingomyelinase (ASM) activity results in the lysosomal storage disorder, Niemann-Pick disease (NPD). Historically, two clinically distinct forms of NPD have been associated with this enzyme deficiency: the infantile, neuronopathic Type A, and the non-neuronopathic Type B. However, recent research has revealed a wide spectrum of disease among patients with ASM deficiency, including the identification of some patients with later onset and less severe neurological involvement. In addition, at least one manifestation of ASM deficiency, very low HDL-cholesterol, is often found in carrier (heterozygous) individuals. It has been suggested previously that the ASM gene locus (*SMPD-1*), located on the short arm of chromosome 11 (11p15.1-15.4), may be imprinted based on the observation that an individual with Beckwith-Weidemann syndrome who inherited two paternal chromosome 11's also had low ASM activity and hepatosplenomegaly (Rethy *et al.*, Path. Oncol. Res., 2000). We therefore examined the expression of *SMPD-1* mutant alleles in several patients and carriers with ASM deficient NPD. We observed preferential expression of the maternal allele, and found that in some families the disease phenotype could be correlated with the severity of the mutation carried on the maternal chromosome. We also observed in some carrier individuals that low HDL-cholesterol could be correlated with maternal inheritance of the mutant allele. Thus, we propose that the variation in clinical findings among ASM deficient NPD patients and carriers may be partially explained by genomic imprinting at the *SMPD-1* locus.

Mutation analysis in *ABCC6* reveals no evidence for dominant inheritance of Pseudoxanthoma Elasticum (PXE).

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In 1972 British families were reported to show dominant inheritance of PXE. Mutations in the *ABCC6* gene cause PXE. During our mutation screen of *ABCC6* in 170 PXE chromosomes from 81 families we also analyzed a subset of 9 families with the occurrence of PXE in more than 1 generation or in first degree cousins suggesting an apparent dominant mode of inheritance of the disease. Familial haplotype analysis did not identify affected sib pairs as heterozygotes at the gene locus. This was confirmed by mutation analysis that showed all affecteds to carry two allelic *ABCC6* mutations in accordance with a recessive molecular inheritance mechanism. The transmission of the disease from an affected parent to the next generation always required two parental mutant allelic variants independent of the clinical disease status of each parent. This proves pseudo-dominance as the relevant inheritance mechanism. Moreover, affecteds of 93 % (67 out of 72) of the families with PXE in a single generation are either homozygous for the same, or compound heterozygous for 2 mutations. The remaining 5 families with only one uncovered mutation show allelic compound heterozygosity for the cosegregating PXE haplotype. Despite other previous clinical and molecular claims our results show only evidence for recessively inherited PXE. This has profound consequences for the genetic counseling of families with PXE.

Refinement of the Cerulean Cataract Type 1 Locus on 17q25.3 to a 0.5 Mb Critical Region. *M.B. Gorin^{1,3}, B.W. Rigatti¹, F.Y. Demirci¹, S.R. Clarke¹, T.S. Mah¹, D.E. Weeks^{2,3}, R.E. Ferrell³*. 1) Dept Ophthal, Univ Pittsburgh, Pittsburgh, PA; 2) Dept Biostatistics, Univ Pittsburgh, Pittsburgh, PA; 3) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA.

Cerulean cataracts (CCA) are autosomal dominant, juvenile-onset hereditary cataracts that are noteworthy for tiny blue or white opacities, predominantly in the lens cortex. The CCA1 locus was originally mapped in a single large family between microsatellite markers D17S802 and D17S836 based on 6 recombinations. We have redefined that interval by identifying additional family members (now totaling 137 members) and evaluating new polymorphic markers. These new markers (microsatellites and SNPs) were evaluated by using a combination of ABI genotyping, DHPLC (Transgenomic Wave), RFLP, and direct PCR sequencing techniques.

At this time, we have established a narrower critical interval of 0.5 Mb. In addition to 7 known microsatellite markers, 10 of the 18 novel microsatellite markers and 16 of the 22 SNPs have been found to be informative in this family. Selection of potential candidate genes within the critical interval has been based on their expression in lens by using the NEIBank lens cDNA database, NCBI UniGene expression information and confirmation by PCR in human lens cDNA library. Thirty-four candidate genes interspersed within the original 4.7 Mbp region of 17q25.3 were evaluated for expression in human lens and for mutation analysis. All exons and splice junctions were screened for mutations by direct PCR sequencing that revealed no evidence of disease-causing variations. Within our new critical region, there are only 15 known or putative genes that have not yet been screened. Of these, 9 show evidence of lens expression. Testing of these genes is currently underway as well as the evaluation of additional SNPs to further refine the critical region and the testing of the remaining 6 genes for expression in the lens cDNA library. Elucidation of the causative gene for CCA1 is likely to identify a novel gene whose role in lens biology has not been previously recognized and which may yield new insights into the pathogenesis of hereditary cataracts.

Four Additional Missense Mutations in *VEGFR3* Underlying Primary Congenital Lymphedema (Milroy disease).

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Primary lymphedema is an excessive fluid accumulation in subcutaneous tissues due to idiopathic failure of lymphatics. Genetic studies have identified specific genetic mutations causing primary lymphedema and lymphedema-associated disorders. Mutations in vascular endothelial growth factor receptor 3 (*VEGFR3*), a transmembrane tyrosine kinase receptor, cause primary congenital lymphedema (PCL) or Milroy disease (MIM 153100) (Irrthum et al., 2000; Karkkainen et al., 2000). Lymphedema-distichiasis syndrome (LD; MIM 153400) has been shown to be caused by mutations in the *FOXC2* gene, a member of the forkhead/winged-helix family of transcription factors (Fang et al., 2000). The most recent studies have identified mutations in the *Sry*-related HMG box containing transcription factor gene *SOX18*, to cause lymphedema associated with hypotrichosis and telangiectasia (HLT; MIM 607823) (Irrthum et al., 2003). To date, thirteen mutations in *VEGFR3* have been identified causing PCL (Irrthum et al., 2000; Karkkainen et al., 2000; Evans et al. 2003). In this study, we present four novel mutations in the *VEGFR3* gene underlying PCL. The mutations were found to co-segregate with lymphedema in the families. All of the mutations were missense mutations causing amino acid substitutions in the kinase domain I or II of the receptor. None of the mutations were present in control individuals. We predict that these mutations result in a defective receptor due to the role of the kinase domain of *VEGFR3* for its catalytic activity. (<http://www.icp.ucl.ac.be/vikkula>) (vikkula@bchm.ucl.ac.be).

Novel Tunisian mutations in mucopolysaccharidoses IH and IVA. *S. Laradi*^{1,2,3}, *M. Erazo*¹, *A. Haj Khelil*², *J. Shabber*¹, *S. Ferchichi*^{2,3}, *A. Kassab*³, *S. Ben Mdalla*³, *M. Chaabouni*⁴, *T. Jraad*⁵, *H. Soua*⁶, *L. Chkioua*³, *H. Bouzidi*^{2,3}, *R. Taalouche*^{2,3}, *H. Zaglaoui*^{2,3}, *B. Tabarki*³, *A. Miled*^{2,3}, *T. Tukul*¹, *R.J. Desnick*¹. 1) Mount Sinai School of Medicine, New York, NY; 2) Faculty of Pharmacy, Monastir, Tunisia; 3) F Hached Hospital, Sousse, Tunisia; 4) H Chaker Hospital, Sfax, Tunisia; 5) Msaken Hospital, Tunisia; 6) T Sfar Hospital, Mahdia, Tunisia.

Mucopolysaccharidoses (MPS) types IH (Hurler syndrome) and IVA (Morquio A) result from the defective activity of -L-iduronidase (IDUA) and N-acetylgalactosamine-6-sulfatase (GALNS), respectively. Both diseases have severe and milder phenotypic subtypes. Three unrelated severe MPS IH patients and four severe MPS IVA patients from Central and Southern Tunisia were screened for DNA mutations. Each of the exons and intron-exon junctions of IDUA or GALNS were sequenced after PCR-amplification from genomic DNAs. In the MPS IH patients, two novel (3904delTinsGAACA in exon 13 and I270S in exon 7) and two previously reported (P533R and R628X) IDUA mutations were detected. The novel missense mutation I270S was found in a patient who also had the nonsense mutation R628X on the other allele. In MPS IVA, three patients were homozygous for a novel G to A transition in the conserved donor splice site in intron 1 (GACgtGACat; designated IVS1^{+1G-A}). The fourth patient was homozygous for a G to C transversion in exon 1 predicting a glycine to arginine missense mutation (G66R). Thus, identification of these mutations should facilitate prenatal diagnosis and counseling for MPS IH and MPS IVA in Tunisia where a very high rate of consanguinity still exists.

Characterization of New *SDHD* Mutations in Sporadic and Familial Head and Neck Paraganglioma from Belgium. A. Persu¹, V. Grégoire², J.-F. De Plaen¹, P. Garin⁴, M. Hamoir³, M. Vikkula⁵. 1) Nephrology Dept and; 2) Radiotherapy Dept and; 3) Otolaryngology Dept, Clin Univ St-Luc, Brussels; 4) Otolaryngology Dept, Clin Univ de Mont-Godinne (UCL), Yvoir; 5) Lab of Human Molecular Genetics, Christian de Duve Institute & Université catholique de Louvain, Brussels, Belgium.

Head and neck paraganglioma (PG) are hypervascular tumors derived from the neural crest. Mutations of the *SDHD* gene, which encode a subunit of complex II of the mitochondrial respiratory chain are at the origin of most familial cases of PG and a minority of apparently sporadic pheochromocytoma. We recruited all patients with PG seen in the main Academic Centers in Belgium from May 2003 to May 2004. Written informed consent, standardized clinical information and blood samples for DNA extraction were obtained. Screening of the coding parts of *SDHD* was performed by SCCP and heteroduplex analysis, followed by sequencing whenever a shift was observed. *SDHD* screening was performed in 31 index patients including 26 patients without familial history of PG and 5 patients with at least one other affected family member. In the latter, recruitment was further extended to available family members. Familial and sporadic cases differed by mean age at diagnosis (30.09.6 vs. 53.515.7 years), proportion of bilateral (78% vs. 12%), multiple (67% vs. 0) relapsing (56% vs. 15%) or ectopic tumours (44% vs. 0%) and prevalence of *SDHD* mutations (80.0% vs. 7.7%). Five different mutations (3 frameshift mutations leading to premature stop codon, one splice site mutation and one substitution) were found in 6 different patients including 4 familial cases and 2 apparently sporadic cases. Four of them were not described previously. Interestingly, the single family for which no *SDHD* mutations was found was characterized by a particularly severe phenotype with rare manifestations including hepatic, vertebral and mediastinal localizations. In this pedigree, screening for mutations in the coding parts of the 2 other genes at the origin of familial PG, *SDHC* and *SDHB*, also proved negative, which suggests further genetic heterogeneity in hereditary PG. (alexandre.persu@nefr.ucl.ac.be) (<http://www.icp.ucl.ac.be/vikkula>).

Preliminary analysis of human chromosome 6q27 locus as modulator of pulmonary disease in cystic fibrosis. I. Evtoushenko¹, D. Markiewicz¹, G. Deng¹, M. Patel¹, R. Dorfman¹, M. Corey¹, M. Tan¹, F. Li¹, A. Sandford³, P. Paré³, P. Durie¹, L-C. Tsui^{2,1}, J. Zielenski¹, and Canadian CF Clinics. 1) Hospital for Sick Children, Toronto, Canada; 2) University of Hong Kong, Hong Kong; 3) iCAPTURE/UBC, Vancouver, Canada.

Several independent studies have demonstrated genetic contribution to pulmonary function (PF). The presence of a major gene locus influencing PF has been recently reported on human chromosome 6q27 (Framingham Heart Study). The strongest linkage and association was documented for the Forced Expiratory Volume in 1 sec (FEV1) using short tandem repeat (STR) markers located in the region. To test for the potential modulating effect of the 6q27 locus on the severity of pulmonary disease in cystic fibrosis (CF) patients we have initiated an association study for selected STR markers in this region. Up to 381 CF families ascertained in the Canadian CF Modifier Gene Project were genotyped for three STR markers from the 6q27 region: D6S281, D6S1027 and D6S1693. The most recent patient PF measurements were adjusted for age, height and gender (percent predicted values; FEV1PP). Standard deviation scores of FEV1PP values were calculated based on a reference population of patients homozygous for deltaF508. Association was tested using the Family Based Association Tests (FBAT) software. Parametric FBAT analysis of association between the spirometric measurements and STR markers was performed using different models (additive, recessive and dominant). The genotype data obtained for these markers demonstrated allelic frequencies similar to those previously published. At present, our preliminary FBAT analysis shows a significant association only for D6S281 with FEV1PP ($p=0.01$) in the dominant model. The same marker was associated in the Framingham Heart Study. We continue the testing in a larger study population to verify the 6q27 region as a potential modifier locus of the pulmonary phenotype in CF. In addition, candidate genes in the region of the associated marker are also being investigated. (Supported by the Genome Canada and Canadian CF Foundation).

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preliminary analysis of human chromosome 6q27 region as a potential modifier locus for pulmonary disease in cystic fibrosis. *I. Tevtoushenko¹, D. Markiewicz¹, G. Deng¹, M. Patel¹, R. Dorfman¹, M. Corey¹, M. Tan¹, F. Li¹, L. Sun³, A. Sandford⁴, P. Pare⁴, P. Durie¹, L.C. Tsui^{2,1}, J. Zielenski¹, participating Canadian CF Clinics Directors.* 1) Genetics and Genomic Biology, Hospital for Sick Children, Toronto, Canada; 2) University of Hong Kong, Hong Kong; 3) University of Toronto, Toronto, Canada; 4) iCAPTURE/UBC, Vancouver, Canada.

Background: The contribution of genetic factors to pulmonary function has been investigated in several independent studies. Heritability of these measures has been estimated at around 40%. Evidence for a major gene locus influencing pulmonary function has been recently reported on human chromosome 6q27 by the Framingham Heart Study. Both linkage and association to FEV1 and FVC were documented for short tandem repeat (STR) markers in the region. We have initiated an association study for selected STR markers in the 6q27 region to test for a potential modifying effect on pulmonary function in CF. Methods: Available trios (CF patient + parents) ascertained by the Canadian CF Modifier Gene Study were genotyped for 3 STR markers selected from the 6q27 region: D6S281 (N=381), D6S1027 (N=180), and D6S1693 (N=149). The most recent spirometric measurements (FEV1 and FVC) submitted by participating Canadian CF Clinics were adjusted for age, height and gender using standard equations (percent predicted FEV1 and FVC).

Golabi-Ito-Hall syndrome results from a missense mutation in the WW domain of the PQBP-1 gene. *F.E. Abidi¹, L. Holloway¹, H. Lubs¹, A. Meindl², R.E. Stevenson¹, C.E. Schwartz¹.* 1) JC Self Research Institute, Greenwood Genetic Center, Greenwood, South Carolina, USA; 2) Department of Medical Genetics, Ludwig-Maximilians-University, Munich, Germany.

Golabi-Ito-Hall syndrome is characterized by X-linked mental retardation (XLMR), microcephaly, short stature and ectodermal manifestations (brittle scalp hair, nail hypoplasia, cutis marmorata, and large maxillary central incisors). The clinical features of microcephaly and short stature are associated with other XLMR syndromes such as the Renpenning syndrome and the Sutherland-Haan syndrome. Recently, mutations in the PQBP-1 gene have been reported in both these syndromes. Based on this observation, analysis of the PQBP-1 gene in a member of the original family with Golabi-Ito-Hall syndrome was undertaken. A missense mutation was identified which changes the conserved tyrosine residue in the WW domain at position 65 to a cysteine (p.Y65C). This finding is unique as it is both the first report of a missense mutation in PQBP-1 and the first mutation identified in the WW domain of the gene.

The WW domain has been shown to play an important role in the regulation of transcription by interacting with the PPxY motif found in transcription factors. The p.Y65C mutation may affect the proper functioning of the PQBP-1 protein as a transcriptional co-activator. The five mutations previously reported cause premature truncation of the PQBP-1 protein. Three of them result in a protein lacking part of the DR/ER repeat in the PRD domain as well as the entire NLS (nuclear localization signal) and the C2 domains. The DR/ER stretch is involved in transcriptional control by binding to the poly Q region of BRN2 which inhibits transcription. The other two mutations affect neither the PRD domain nor the NLS domain. Instead, they give rise to a truncated protein which partially or completely lacks the C2 domain, which maybe involved in signal transduction or membrane trafficking. It is interesting that the ectodermal manifestations observed in the Golabi-Ito-Hall syndrome have not been reported in the families with truncating mutations.

ATRX syndrome : Novel mutations in the helicase domain. *C. Badens¹, F. Giuliano², A. Verloes³, C. Mouradian¹, P. Malzac¹, N. Philip¹, N. Levy¹.* 1) Department of Genetics, Hopital de la Timone, Marseille, France; 2) Departments of Genetics, Hopital l'Archet 2, Nice , France; 3) Department of Genetics, Hopital Robert Debre, Paris, France.

The main features of the ATR-X syndrome are profound mental retardation, characteristic facial dysmorphism and -thalassemia. Although this latter feature is not constant, it led to the initial identification and description of this syndrome. The gene involved in this syndrome, designated ATRX, maps to Xq13.3 and codes for a zinc finger helicase . Since its identification, more than 70 mutations have been described in 150 families. Correlation between phenotype and genotype is not straight forward but so far, the Zn finger domain, one of the functional domain, has been much often investigated than the other regions, especially the helicase domain. As a result, 80% of the mutations described are localised in the Zn Finger domain. In order to determine if mutations in other parts of the gene have been under-investigated, we planned to perform systematic sequencing of the ATRX gene in a group of 30 patients selected as follow : 1-Characteristic facies, 2- unbalanced X inactivation in the patients mother, 3-no mutation in the Zn Finger domain. So far, we investigated the region from exons 16 to 35 which includes the helicase domain. Three (10%) new missense mutations were found in exons 19, 26 and 30 (respectively G1676A, L2022F and R2178W), all situated in highly conserved regions. Although 20% of the targeted region remains to be analysed, mutations in the 3part of the gene seem to be less frequent than mutations in the Zn finger domain, representing 20% of all ATRX mutations identified in our laboratory. Beside the molecular study, exhaustive clinical data are collected, including IQ determination, in the patients currently investigated and in patients previously characterised with mutation in the Zn finger domain. This will permit to determine if a specific phenotype (especially mild mental retardation) is associated with mutations in the 3 part of the gene.

Kainic acid seizures in the fragile X knock out mouse and reduced GABA-A receptor expression. *C. Dobkin¹, A. El Idrissi³, X-H. Ding¹, E. Trenkner², R. Bauchwitz⁴*. 1) Dept Human Genetics, NYS Inst Basic Research, Staten Island, NY; 2) Developmental Cell Biology, NYS Inst Basic Research, Staten Island, NY; 3) Department of Biology, College of Staten Island/CUNY, Staten Island, NY; 4) St. Luke's-Roosevelt Institute of Health Sciences, Columbia University, NY, NY.

One feature of the fragile X mental retardation syndrome is an increased prevalence of childhood seizures. The fragile X mouse model for this disorder has been shown to have an increased susceptibility to audiogenic seizures. We found that the fragile X mouse also has increased susceptibility to limbic seizures induced pharmacologically by peripheral injection of kainic acid (KA). Congenic FVB/N as well as congenic C57BL/6 fragile X mice both show increased susceptibility to KA seizures quantified by seizure duration and by excitotoxic cell death in the CA3 region of the hippocampus. We also found that introduction of the human fragile X gene, FMR1, into the FVB/N fragile X mice essentially reversed the increased seizure susceptibility induced by the knock out of the endogenous Fmr1 gene. To investigate the neuropathogenesis of increased seizure susceptibility, we examined GABA-A receptor expression in the FVB/N fragile X mouse. Western blot analysis indicated that expression of the GABA-A receptor beta subunit (GABAR β) was reduced in the cortex, hippocampus, diencephalon and brainstem in adult male fragile X mice. Immunohistochemical analysis of brain sections suggested a reduction in GABAR β immunoreactivity. These results imply that the absence of Fmrp leads to significant alterations of the GABAergic system that could account for the increased seizure susceptibility of the fragile X mouse and may be relevant to the seizures and the abnormal behaviors in the fragile X syndrome.

A novel candidate gene for X-linked non syndromic mental retardation. A. Dubos¹, H. Dollfus², F. Abidi³, N.L. Tang⁴, S. du Manoir¹, S. Pannetier¹, C.E. Schwartz³, A. Hanauer¹. 1) Institut de Genetique et de Biologie Moleculaire et Cellulaire, CNRS/INSERM/ULP, BP10142, 67404 Illkirch Cedex, France; 2) Service de Genetique Medicale, Hopitaux Universitaires de Strasbourg, Avenue Moliere, 67098 Strasbourg Cedex, France; 3) J C Self Research Institute, Greenwood Genetic Center, Greenwood, SC, USA; 4) Department of Chemical Pathology, Faculty of Medicine, The Chinese University of Hong Kong, Kong Kong.

Non syndromic X-linked mental retardation (MRX) is defined by an X-linked inheritance of low IQ (<70) and adaptive skills limitations without other clinical features. Eighteen MRX genes have been identified so far, while more than 50 are expected. To identify novel MRX genes, we have studied female patients presenting mental retardation and balanced X:autosome translocations. After detection of the X breakpoint spanning clone by fluorescence *in situ* hybridization (FISH), we searched *in silico* for genes in the breakpoint region. In one patient with mild mental retardation and a (X:19) translocation, we identified a gene, KIAA1202 disrupted by the X breakpoint. Furthermore, a serine to leucine amino acid change (S1089L) was found in a Brazilian family after KIAA1202 mutation screening in a panel of 18 X-linked mental retardation families from South and North America. S1089L change segregated with the disease in the family and was not found in 300 control individuals. Northern-blot analysis in human revealed a predominant transcript in all tissues and brain regions tested with high expression in medulla, putamen and occipital, frontal and temporal lobes. RT-PCR experiments in mouse showed KIAA1202 mouse homolog expression during development (E7,5 to E16,5) and in all brain regions tested with highest expression in brain stem, striatum and cerebellum. KIAA1202 belongs to the Apx/APXL/Shroom protein family. Apx is associated in a macromolecular complex to the epithelial sodium channel (ENaC) and Shroom regulates cytoarchitecture during neurulation. Moreover, KIAA1202 contains a PDZ domain, a motif also present in DLG3, recently identified as an important contributor gene in MRX. Our results suggested that KIAA1202 is a good candidate gene for mental retardation.

A novel in-frame deletion in ARX is associated with lissencephaly with absent corpus callosum and hypoplastic genitalia. *R.C. Rogers, S. Bhat, K.R. Holden, A.K. Srivastava.* J. C. Self Research Institute of Human Genetics, Greenwood Genetic Center, Greenwood, SC.

Aristaless-related homeobox (ARX) gene mutations cause several X-linked neurodevelopmental disorders that include lissencephaly with abnormal genitalia (XLAG), syndromic and nonsyndromic mental retardation, dystonia, and epilepsy. We identified an infant who had multiple neonatal tonic-clonic seizures. Initial exam showed unusual cranial shape with posterior sloping and flattening of the frontal skull and hypoplasia of the external genitalia, with a small penis and undescended testes. The anterior fontanel was soft and measured 4 cm x 4 cm. There was mild splitting of the sutures. The brain magnetic resonance imaging scan demonstrated lissencephaly with pachygyria and agenesis of corpus callosum (ACC). His karyotype was determined to be 46, XY. Mutations of the DCX gene in this infant were ruled out. We analyzed the ARX gene and identified a novel 9-bp deletion at nucleotide 996-1001 (c.996/1001_1001/1009 del9) in exon 2. The alteration is predicted to result in an in-frame deletion of 3 amino acids (p.T333/T334_T335/F336 del.) in the ARX homeodomain. The change was found to be absent in 368 control male individuals. Whether this novel alteration is a rare polymorphism can not be completely excluded. However, our findings are consistent with a previous report in which nonconservative missense mutations in the homeodomain of the ARX were found in XLAG patients with a milder phenotype (lissencephaly with hypoplastic male genitalia rather than ambiguous genitalia). Our results suggest that the novel in-frame deletion in the ARX homeodomain is likely to have a pathogenic role as an etiology for lissencephaly with ACC and hypoplastic genitalia.

Mutation screening of *MECP2* gene causing Rett syndrome. D. Zahorakova¹, R. Rosipal¹, J. Hadac², M. Misovicova³, A. Zumrova⁴, L. Pospisilova¹, V. Bzduch⁵, J. Zeman¹, P. Martasek¹. 1) Department of Pediatrics, 1st Faculty of Medicine, Charles University, Prague, Czech Republic; 2) Department of Child Neurology, Thomayer University Hospital, Prague, Czech Republic; 3) Department of Clinical Genetics, Martin University Hospital, Martin, Slovakia; 4) Department of Child Neurology, University Hospital Motol, Charles University, Prague, Czech Republic; 5) 1st Department of Pediatrics, Comenius University, Bratislava, Slovakia.

Rett syndrome (RTT) is a severe X-linked neurodevelopmental disorder, affecting almost exclusively females. It is caused by mainly de novo mutations in ubiquitously expressed methyl-CpG-binding protein 2 gene (*MECP2*). Methyl-CpG-binding protein 2 (MeCP2) binds specifically to methylated DNA and plays an important role in gene silencing. Mutations in *MECP2* lead to decrease/loss of MeCP2 function. More than 200 mutations have been identified in *MECP2*, including missense, nonsense, deletions and insertions. We report mutation analysis of 67 girls with clinical diagnosis of classic RTT from Czech and Slovak Republics and prenatal diagnosis in three families with RTT patient. Genomic DNA was used to amplify the coding sequence and exon/intron borders of *MECP2*. Products were examined by RFLP and sequencing. The analysis revealed 20 different mutations in 50 patients. 27 patients had missense mutations (R106W, R133C, S134C, K135E, T158M, P302S, R306C), 15 patients carried nonsense mutations (Y141X, R168X, S204X, R255X, R270X, R294X), 7 had frameshift mutations (K286fs, G269fs, S355fs, H372fs, P385fs, P388fs) and one had in-frame deletion S357. 3 mutations have not been previously published: a missense mutation P302S and deletions S355fs and S357. The frequency of mutation T158M compared to other databases (e.g. RettBASE 9.25%) has been much more higher in our patients (22%). Prenatal diagnoses were performed in 3 families with patients carrying mutations R168X, S204X and P388fs. No mutations in fetal DNA were found. Our results show molecular heterogeneity in Czech and Slovak patients with RTT, facilitate the RTT diagnosis at the molecular level, and provide insight into the molecular pathology of RTT. Supported by grant GAUK8/04.

ETHE1 mutations are a prevalent cause of Ethylmalonic Encephalopathy. *M. Zeviani¹, E. Briem¹, E. Lamantea¹, P. Rinaldo², E. Christensen⁸, P. Dickinson³, F. Ezgu⁴, M. Owaidha⁵, L. Heberle⁶, B. Abu-libdeh⁷, V. Tiranti¹.* 1) Dept Molecular Neurogen, Inst Nazionale Neurologico, Milano, Italy; 2) Mayo Clinic, Rochester, MN; 3) Harbor-UCLA Medical Center, Torrance, CA; 4) Gazi University Hospital, Ankara, Turkey; 5) Al-Jahara Hospital, Kuwait; 6) Al-Sabah Hospital, Kuwait; 7) Al-Quds University, Jerusalem; 8) Dept. of Clinical Genetics, Juliane Marie Centre, Rigshospitalet, Copenhagen, Denmark.

The identification of nuclear disease genes is a hot issue in mitochondrial medicine, but is hampered by the clinical and genetic heterogeneity of mitochondrial syndromes and the scarcity of informative families. The development of new bioinformatics tools and the completion of the human genome project, together with traditional strategies, can help progress in this area. An example is the recent discovery of the gene responsible for Ethylmalonic Encephalopathy (EE). EE is an infantile, fatal metabolic disorder, characterized by symmetrical lesions in the basal ganglia and brainstem, diffuse microvasculopathy, hyperlactic acidemia and high levels of ethylmalonic acid (EMA) in the body fluids. The 625G>A SNP in the short-chain acylCoA dehydrogenase (SCAD) gene was proposed as a co-factor in the etiology of EE, but this hypothesis has remained controversial for many years. Using an integration of physical and functional genomic data sets, we identified ETHE1 as a new gene, on chromosome 19, which is responsible for this disease. To investigate the prevalence of ETHE1 mutations and the 625G>A SNP in EE, we analyzed 28 patients with typical EE and 9 patients with non-EE EMA aciduria. Sequence analysis revealed 14 loss-of-function mutation and 7 missense mutations in 27 out of 28 typical EE cases. No ETHE1 mutations were identified in non-EE EMA aciduria cases. No difference in the frequency of the 625G vs. the 625A SCAD alleles was found in the ETHE1 mutants and in 53 control individuals. The 625A allele was significantly over-represented in non-EE EMA aciduria. Our data indicate that mutations of ETHE1 are the only responsible for the high level of excretion of ethylmalonic acid in this condition.

Rapid and reliable multiplex-PCR assay for deletions causing hereditary persistence of fetal hemoglobin

(HPFH). *U. Bhardwaj*^{1,2}, *L.L. McCabe*^{1,2}, *E.R.B. McCabe*^{1,2}. 1) Depts of Pediatrics and Human Genetics, David Geffen School of Medicine at UCLA, LA, CA; 2) Mattel Children's Hospital at UCLA, LA, CA.

The human γ -globin gene locus consists of five developmentally regulated genes 5-GA-3, regulated by two switches: embryonic (ϵ) to fetal (γ) and fetal (γ) to adult (β and δ) globin gene. Occasionally failure of normal hemoglobin switching from fetal to adult genes leads to persistent production of fetal hemoglobin (Hb F) into adulthood, resulting in HPFH. Clinically HPFH is a benign condition; however, it can reduce the phenotypic consequences for individuals homozygous for α -thalassemia or sickle cell disease. In addition, for newborn screening, neonates co-inheriting an HPFH deletion on one chromosome with the S allele on the other can be misinterpreted as homozygous for sickle cell disease when polymerase chain reaction (PCR) amplification is used. So far, eight deletions of variable size and position are known to be associated with HPFH in certain populations. Therefore, our purpose was to develop multiplex-PCR assay to detect these deletions in a single tube to facilitate rapid and accurate molecular diagnosis. Multiplex-PCR was performed using a gap-PCR strategy. Primers were designed across the deletion breakpoints of HPFH-1 (Black), HPFH-2 (Ghanaian), HPFH-3 (Indian), HPFH-4 (Italian), HPFH-5 (Italian), HPFH-6 (Vietnam), HPFH-7 (Kenya) and SEA-HPFH (Southeast Asia). The technique was standardized on DNA samples with known deletions (NIGMS Human Mutant Cell Repository) or cloned breakpoints. For two deletions, HPFH-3 and HPFH-7, we generated positive controls by cloning ligated telomeric and centomeric breakpoint regions. The breakpoint region of these controls was confirmed by sequencing. The multiplex-gap-PCR approach was tested on samples with known deletions by using eight pairs of primers bridging each breakpoint in a single tube. We were able to amplify a distinct deletion-specific product for all eight deletions without any false positive or negative amplification. We conclude that this multiplex-PCR strategy provides the most rapid and accurate diagnosis for the deletions of the γ -globin cluster causing HPFH.

Factor IX germline mutations in early embryo: Preferential occurrence of G:C>A:T non-CpG transitions. *W.A. Scaringe¹, J. Drost^{1,2}, X. Li¹, A. Halangoda¹, K. Hill^{1,3}, R. Ketterling⁴, C. Kasper⁵, S.S. Sommer¹.* 1) Departments of Molecular Genetics and Molecular Diagnosis, City of Hope National Medical Center, Duarte, CA, USA; 2) Department of Biological Sciences, Louisiana State University, Baton Rouge, LA, USA; 3) Department of Biology, The University of Western Ontario, London, ON, Canada; 4) Department of Pathology, Mayo Clinic/Foundation, Rochester, MN, USA; 5) Orthopaedic Hospital, Los Angeles, CA, USA.

Hemophilia B is a model for analyzing recent germline mutations in humans. For 91 individuals identified as the mutation origin, leukocyte DNA sequences of *F9* were analyzed directly by genomic sequencing. Somatic mosaicism for the causative mutation was identified in six females and three males with 5% to 70% of the leukocytes detected as mutant. One additional origin female transmitted the mutation to two offspring although the mutation was not detected in her leukocytes. Six of these ten defined mosaics had G:C>A:T transitions at non-CpG dinucleotides. Three of these six were at the G of CTG sites and another was adjacent to CTG. The preferentially embryonic occurrence of this *F9* mutation type over all other types is significant ($p=0.002$). This suggests that non-CpG G:C>A:T transitions occur preferentially in early embryogenesis, an intriguing observation given the recent report of a high frequency of non-CpG methylation in embryonic stem cells.

OPA3 gene mutations are responsible for autosomal dominant optic atrophy and cataract (ADOAC). D.

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Hereditary optic atrophy is a heterogeneous group of genetic disorders in which several modes of inheritance have been described. The most common forms are autosomal dominant optic atrophies (ADOA, OMIM 165500) and Leber's hereditary optic neuropathy (LHON, OMIM 53500). OPA1, encoding for a mitochondrial protein, is currently the only gene identified in ADOA and is involved in 60-80% of the cases. Here we report that OPA3, a gene until now implicated in type III methylglutaconic aciduria, an autosomal recessive syndromic optic atrophy (OMIM 258501) is also responsible for ADOA with cataract (ADOAC). OPA3 was directly sequenced as a candidate gene in two unrelated families affected with ADOAC of whom 11 affected individuals and 10 healthy relatives participated in the study. Two different mutations in exon 2 of OPA3 were found in patients with ADOAC. The 277G-A (G93S) mutation was present in one family, and the 313C-G (Q105E) mutation in the other. Both mutations segregated with the disease in each family and were absent in healthy relatives and in 400 control chromosomes. Since OPA3 encodes a mitochondrial protein of unknown function, the possible mitochondrial dysfunction in skin fibroblasts from a patient affected with ADOAC was investigated. The respiratory chain, the mitochondrial membrane potential and the organisation of the mitochondrial network showed no abnormalities. However, the fibroblasts were highly susceptible to sautosporine-induced apoptosis. The results indicate that OPA3 is responsible for autosomal dominant OA as well as autosomal recessive OA. Thus, along with autosomal dominant optic atrophy (mutations in OPA1) and Lebers hereditary optic neuropathy (mutations in mtDNA), ADOAC (mutations in OPA3) is yet another form of hereditary optic atrophy involving a mitochondrial inner membrane protein.

Cellular models for Friedreich Ataxia uncover unexpected consequences of frataxin deficiency. *H. Seznec¹, N. Carelle¹, D. Simon¹, L. Reutenauer¹, P. Rustin², M. Koenig¹, H. Puccio¹.* 1) Molecular Pathology, IGBMC, Illkirch, France; 2) INSERM U393, Hopital Necker-EM, Paris, France.

Friedreich ataxia (FRDA), the most common autosomal recessive ataxia, is defined by a progressive sensory ataxia and cardiomyopathy. The disease is due to a severely reduced level of frataxin (fxn), a mitochondrial protein involved in Fe/S cluster and heme synthesis. The mechanisms leading to Fe/S enzyme deficiency, mitochondrial iron deposits, and impaired oxidative stress defense are still controversial. Deciphering the molecular mechanisms and finding therapeutics for FRDA would be facilitated by the availability of cellular models. Until now, cellular models for FRDA rely on an exogenous oxidative stress to produce a phenotype. Therefore, although these models reproduce part of the pathophysiology due to oxidative insult, none present the FRDA features observed in patient tissues. In addition, the nature of these models make them unsuitable for pharmacological screening procedures. We therefore urgently need to generate cell models that faithfully and spontaneously reproduce the FRDA features. We generated fxn knock-out and knock-down immortalized cell lines. Total fxn deficiency leads to cell death in proliferating cells, however in vitro myogenic differentiation is not altered. These data indicate that the absence of fxn is more deleterious in dividing cells than in post-mitotic cells, even rich in mitochondria. To circumvent this lethality, we generated a fxn knock-down cell lines by using a ribozyme targeted against fxn. Several clones show a significant decrease in fxn expression (9,6% residual level), a growth retardation, and a decrease in aconitases activity (Fe/S enzyme). Surprisingly, the ribozyme cellular models show decreased sod2 expression (-30%), without oxidative stress, and no iron metabolism alteration. These unexpected observations, also found in the mouse models, underlined that these knock-down cellular models reproduce alterations closely linked to fxn deficiency. Therefore, these novel models are the first good tool to decipher the molecular consequences of frataxin deficiency and for large scale drug screening for finding therapeutics for FRDA.

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Development of an ALSIN Knockout mouse model. *H.X. Deng, R. Fu, H. Zhai, E. Soriano, T. Siddique.* Neurology Dept, Northwestern Univ, Chicago, IL.

Amyotrophic lateral sclerosis (ALS) is a progressive paralytic disorder caused by degeneration of the upper motor neurons in the motor cortex and lower motor neurons in the brainstem and the spinal cord. We previously linked the recessive form of juvenile ALS type 3 (ALS2) to chromosome 2q3335, and subsequently we reported that a differentially spliced gene encoding short and long forms of a protein, which we named alsin, is mutated in both juvenile ALS and juvenile primary lateral sclerosis (JPLS). This gene has two transcriptional forms with two distinct poly(A) signals. The short form has 4 exons and the long form has 34 exons, respectively, with the first 4 exons shared by both forms. The gene products, which we called alsin, are two distinct proteins with the short one being 396 amino acids and long one being 1657 amino acids in size, and the short form shares 371 amino acids with the long form at the N-terminus (Yang et al, 2001). To investigate the physiological function of alsin and the pathogenic mechanism by which loss of alsin leads to ALS or PLS, we constructed a targeting vector that was designed to replace exon 4 and a part of exon 3 with neo- cassette. We identified two positive ES cells with right targeting events and alsin gene-targeted mice were developed from these ES cells. The homozygous mice develop motor deficit from around 1 year old. Progression of the disease is very slow, as that seen in humans. Axonal degeneration is present in cortex, cerebellum and spinal cord.

Chromatin remodeling and transcriptional alterations in polyglutamine expansion diseases. *D. Devys¹, D. Helmlinger¹, S. Hardy², G. Abou-Sleymane¹, Y. Trottier¹, L. Tora², J.L. Mandel¹.* 1) Dept Molecular Pathology, IGBMC, Illkirch, France; 2) Dept of Transcription IGBMC, Illkirch, France.

Spinocerebellar ataxia type 7 (SCA7) belongs to a group of progressive neurodegenerative diseases, caused by an expansion of a polyglutamine tract in the respective disease-causing proteins. Genetic and biochemical studies have shown that polyglutamine pathogenesis arises from a gain of function. We demonstrated that ataxin-7, the protein mutated in SCA7, is the human orthologue of yeast SGF73, recently identified as a new subunit of the Spt/Ada/Gcn5 histone acetylase (SAGA) complex, which is a coactivator required for transcription of a subset of RNA polymerase II-dependent genes. Ataxin-7 is a bona fide subunit of human TBP-free GCN5 histone acetyltransferase-containing complexes (TFTC/STAGA), which are mammalian SAGA-like complexes. Using SCA7 patient cells and our SCA7 transgenic mouse model, we observed that normal and mutant ataxin-7 can equally incorporate into TFTC/STAGA complexes. In parallel, we investigated whether transcriptional dysregulation underlies the retinal dysfunction that we recently described in SCA7 mice. Using Affymetrix chips and quantitative RT-PCR, we observed an early and strong downregulation of numerous genes that are highly and specifically expressed in rod photoreceptors. Finally, these transcriptional alterations were strikingly correlated with a dramatic chromatin remodeling in photoreceptors nuclei, suggesting that downregulation of photoreceptor-specific genes is caused by dysregulation of chromatin-modifying factors. We are thus currently assessing whether the presence of mutant ataxin-7 within the TFTC/STAGA complexes modifies their histone acetyltransferase activity and/or their recruitment at specific promoters. A non-regulated transcriptional activity would result in a gain of TFTC/STAGA function that might provide a specific mechanism for SCA7 retinal pathogenesis.

A mouse model for oculodentodigital dysplasia generated by random mutagenesis. *A.M. Flenniken¹, I. Vukobradovic¹, I. Voronina¹, D. Qu¹, Y. Zhu², G.A. Wood¹, K. Peterson¹, R. Zirngibl¹, N. Anderson¹, J.G. Sled², J. Henderson⁴, C. McKerlie¹, J.E. Aubin¹, W.L. Stanford¹, B.G. Bruneau², G.M. Kidder³, S.L. Adamson¹, L.R. Osborne¹, J. Rossant¹.* 1) Centre for Modeling Human Disease, Toronto, Canada; 2) Hospital for Sick Children, Toronto, Canada; 3) Physiology and Pharmacology, University of Western Ontario, London, Canada; 4) Centre for Bone and Periodontal Research, McGill University, Montreal, Canada.

Oculodentodigital dysplasia (ODDD) is a dominant, multisystem disorder characterized by Type III syndactyly, plus numerous other clinical symptoms with variable penetrance, such as neurodegeneration, craniofacial abnormalities and conductive deafness. More than 30 families with ODDD have been shown to harbour missense mutations of GJA1 (connexin 43) that are predicted to disrupt the proper formation of gap junctions in many tissues. Gja1 gene targeted mouse mutants exhibit a recessive phenotype and die at birth from cardiac malformations making them poor models for the human disease.

We have identified a mouse model for ODDD from a dominant, random chemical mutagenesis screen in mice. The mutant mouse harbors a G60S amino acid substitution and exhibits many of the clinical symptoms of humans with the disorder. These symptoms include syndactyly, absence of the middle phalanx of the fifth digit of the hind limbs, enamel hypoplasia, and abnormalities in cardiovascular function resulting in mild first degree atrioventricular block with some instances of complete heart block as assessed by conscious ECG telemetry.

In addition, we have discovered other phenotypes that have not been reported in humans, such as a reduced bone density and bone mineral composition, and severe bone marrow atrophy of the long bones, which is evident at 25 weeks of age and becomes progressively worse. Immunostaining of tissue sections from the mutant and wildtype mice revealed very few identifiable gap junction plaques containing connexin 43 in heart or ovary. This mouse represents a true model of the human disorder ODDD, and will be invaluable in determining the functional nature of mutations in GJA1.

Osteopontin and kidney stone disease in a mouse model. *H. Tinkel-Vernon, C. Osborne, J. Chen, J. Tischfield, A. Sahota.* Dept Genetics, Rutgers Univ, Piscataway, NJ.

Cell culture studies and animal models of acute renal injury have shown that osteopontin (OPN) inhibits the aggregation of calcium oxalate monohydrate crystals and promotes inflammation at sites of tissue injury. However, the role of OPN in chronic inflammatory kidney stone disease is not well defined. The adenine phosphoribosyltransferase (*Aprt*) knockout mouse, with 2,8-dihydroxyadenine nephrolithiasis, is a well-characterized model for the study of this disease. To better delineate the role of OPN, we generated *Aprt/Opn* double knockout (DKO) mice and examined the kidneys from these and *Aprt* knockout mice using histochemical morphometric, and gene expression techniques. Twelve week old DKO male mice had more kidney stones, increased fibrosis and more markers of inflammation than their *Aprt^{-/-}Opn^{+/+}* counterparts. We attribute this to the stone inhibiting properties of OPN, leading to more stones and more pathological changes in DKO mice. *Aprt* knockout female mice had less pathology than their male counterparts regardless of the *Opn* genotype. However, kidneys from DKO female mice had more pathology than *Aprt* knockout female mice. Interestingly, the DHA stone burden in the two female groups was comparable. We hypothesized that some of the phenotypic differences may be due to OPN causing the up-regulation of inflammation-related genes which are differentially expressed between genotypes and sexes. cDNA array and RT-PCR analyses identified several such genes, including interleukin 15 receptor, interleukin 10 receptor, interleukin 6 signal transducer, MCP-3, and interleukin 2. The expression patterns of these genes and their functions reflect the phenotypic differences seen. Increased expression of IL-15 receptor and interleukin 6 signal transducer in wild type female compared with male mice may explain the severity of disease in males regardless of *Opn* genotype. MCP-3 was most highly expressed in male DKO mice; OPN potentially down regulates this gene and this may explain the severe phenotype seen in DKO male mice. Increased inflammation in OPN deficiency is likely due to the increased stone burden and an up-regulation of pro-inflammatory genes.

Mutation analysis of MECP2A and MECP2B in patients referred for Rett Syndrome. *S.L. Bleoo, P.J. Bridge, A.M. Innes, J.S. Parboosingh.* Medical Genetics, University of Calgary, Calgary, Alberta, Canada.

Rett syndrome is a neurological disorder characterized in females by a period of normal development (6-18 mos.) followed by developmental stagnation and then regression of fine motor and language skills. Patients develop stereotypical hand movements with loss of purposeful hand movements. Although females with Rett syndrome survive into adulthood, the developmental regression results in profound mental retardation. Rett syndrome is caused by mutations in the MECP2 gene, found in Xq28. This gene encodes a transcriptional repressor which, when bound to methylated CpG dinucleotides, affects chromatin remodelling. Originally it was thought that MECP2 involved a single isoform whose translational start site begins in exon 2. This transcript is now referred to as MECP2A. A second isoform, MECP2B was recently identified as a transcript whose initiation codon is in exon 1 but, due to alternative splicing, does not include exon 2. Although most mutations have been identified within exons 3 and 4, two reports have described frameshift mutations in classic Rett patients that affect exon 1. We have investigated 40 patients, 7 male and 33 female, referred through our medical genetics clinic for Rett syndrome for mutations in MECP2A and MECP2B by direct sequencing. We identified 10 mutations in the 33 females and no mutations in the males. All 10 mutations were in exon 4. One was a novel mutation, P388T (MECP2A) or P400T (MECP2B), identified in a two-year old girl. This patient does not appear to have classic Rett syndrome: it is unclear whether she ever had a period of normal development, and she developed no speech or purposeful hand movements. However, our patient does have Rett syndrome features including progressive microcephaly, hand stereotypies, bruxism, growth retardation, and lack of verbal communication. Our laboratory has not confirmed the involvement of MECP2B in Rett syndrome. However, given the documented cases of MECP2B mutations in affected individuals, clinical testing will continue to involve sequencing of exons 1 - 4 as well as multiplex ligation-dependent probe amplification (MLPA) to detect deletions missed by sequencing.

Y-Position Cysteine Substitution in Type I Collagen (1(I) R888C) Is Associated with Type IV Osteogenesis

Imperfecta. W.A. Cabral¹, A. Ashok¹, E. Makareeva², A.D. Letocha¹, N. Scribanu³, S. Leikin², J.C. Marini¹. 1) Bone & Extracellular Matrix Branch, NICHD/NIH, Bethesda, MD; 2) Sect. on Physical Biochemistry, NICHD/NIH, Bethesda, MD; 3) Dept. of Pediatrics, Georgetown Med. Ctr., Washington, D.C.

The most common mutations in type I collagen causing types II-IV OI result in substitution of a glycine in a Gly-X-Y triplet by another amino acid residue. Only one non-glycine substitution in type I collagen has been described; it is an X-position change, 1(I) arg134cys, which causes Ehlers-Danlos syndrome. We have delineated a Y-position substitution in a father and son with mild type IV OI. The 12 yr old proband sustained 4 fractures and has moderate joint laxity. He is osteopenic with L1-L4 DEXA z-score of -1.3. His father had 2 fractures and large-joint hyperextensibility. They have an 1(I) arg888cys substitution in one COL1A1 allele. The CT nt change eliminates an MboI restriction site and heterozygosity was confirmed in gDNA. SDS-urea-PAGE of [³H]-proline labelled steady-state collagen reveals only slight overmodification of the 1(I) monomer band, less than expected for a glycine substitution at this position, and a faint 1(I) dimer. [³⁵S]-cysteine labelling of collagen demonstrated dimers in about 10% of trimers in media and cell layer of fibroblasts. Since a maximum of 25% dimers can occur, dimer formation is inefficient, probably due to decreased proximity of the cysteines in adjacent chains. Immunofluorescence detected no accumulation of mutant procollagen in the ER on ascorbate stimulation, confirming efficient secretion of mutant procollagens from fibroblasts. Differential scanning calorimetry revealed only local helix destabilization. In matrix deposited by cultured fibroblasts, [³H]-proline labelled dimers were seen in immaturely and maturely cross-linked fractions. *In vivo*, proband dermal fibril diameters have a wider range than controls. This Y-position argcys substitution may cause matrix abnormalities by (a) loss of an arginine residue important for staggered helix conformation and interaction with adjacent helices during fibril growth, and/or (b) gain of a reactive SH moiety in this position.

Genetic factors modifying clinical features of dominant retinal diseases: role of polymorphic protein haplotypes

in trans. S.P. Daiger¹, S.P. Shankar¹, A.B. Schindler¹, L.S. Sullivan¹, S.J. Bowne¹, T.M. King², E.W. Daw³, J.R. Heckenlively⁴, E.M. Stone⁵, D.K.H. Wheaton⁶, D.G. Birch⁶. 1) Human Genetics Ctr, Sch Public Health, and Dept Ophthalmol, Univ Texas HSC, Houston, TX; 2) Med Sch, Univ Texas HSC, Houston, TX; 3) MD Anderson Cancer Ctr, Univ Texas, Houston, TX; 4) Kellogg Eye Ctr, Univ Michigan, Ann Arbor, MI; 5) Dept Ophthalmol, Univ Iowa Hosp Clinics, Iowa City, IA; 6) Retina Found SW, Dallas, TX.

Clinical features of dominantly-inherited diseases often differ considerably among affected individuals, even individuals within the same family. To evaluate the role of genetic factors in modifying autosomal dominant retinitis pigmentosa (adRP), we are studying two model systems: adRP caused by an Arg677ter mutation in the RP1 gene, and retinal disease caused by an IVS2+3 AT mutation in the peripherin/RDS gene. The RP1 mutation is found in over 90 members of an extended family with classic symptoms of adRP. Individuals with the RP1 mutation vary in severity, including two non-penetrant individuals. The RDS mutation is present in over 40 individuals from 8 independently-ascertained families with dominant retinopathies. Individuals with the RDS mutation vary in phenotype, with diagnoses of retinitis pigmentosa, pattern dystrophy or central RPE atrophy.

For both mutations, haplotype analysis confirms that the mutation descends from a common ancestor, thus excluding variation in *cis* to the mutation as a factor. Both genes, though, harbor multiple, polymorphic amino acid substitutions, with 3 to 5 distinct, polymorphic protein haplotypes in each case. The haplotypes in *trans* are plausible modifying factors.

In a retrospective study we tested for association between severity (RP1) or phenotype (RDS) and haplotypes in *trans*. Association was evaluated using analysis of variance, a TDT test, and Loki, a Monte Carlo Markov chain approach. For RP1, analysis suggests that a portion of the genetic component of variability maps to the disease locus. Further analysis suggests that RP1 haplotype 3 may play a role in modifying severity. For RDS, preliminary analysis does not support involvement of the RDS haplotypes.

Further molecular characterization of an Alagille Like Syndrome with Autosomal Recessive Inheritance not linked to JAG1 locus. *S. Dyack*^{1, 3}, *M. Cameron*³, *A. Otley*^{1,3}, *W. Greer*^{2,3}. 1) Dept of Pediatrics, IWK Health Centre; 2) Dept of Pathology, QEII Health Sciences Centre; 3) Dalhousie University, Halifax, Nova Scotia, Canada.

Alagille syndrome is an autosomal dominantly inherited syndrome characterized by intrahepatic bile duct paucity and other associated features including cardiac anomalies, vertebral anomalies, a recognizable facial gestalt, and ocular findings. The development of this condition is associated with mutations in the JAG1 gene, located on chromosome 20p. We have identified a consanguineous Native American kindred with five affected individuals with a paucity of the intrahepatic bile ducts and several other features of Alagille syndrome that is inherited in an autosomal recessive fashion. We first initiated linkage analysis for the JAG1 locus, and the disease was neither associated with homozygosity by descent or with a consistent haplotype at the JAG1 locus. Further investigation was initiated for linkage to other known genes in the notch signalling pathway, and no linkage was detected. A genome scan to determine areas of homozygosity by descent is underway. In conclusion we here illustrate a unique family where where an Alagille like syndrome with intrahepatic paucity of bile ducts is inherited in an autosomal recessive fashion and is not linked to the JAG1 locus. Previously only JAG1 has been implicated in the development of Alagille syndrome. Further investigation of this family may confirm genetic heterogeneity in the development of Alagille syndrome.

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Deficient mucus production causes chronic enteritis in the MONTEZUMA mouse model for IBD. *J. Grosse, L. Zeitlmann, B. Schneider, W. Jagla, P. Wabnitz, A. Marquardt, J. Laufs, A. Popp, K. Dembowski, M. Nehls.* Ingenium Pharmaceuticals, Martinsried, Germany.

Ingenium uses random mutagenesis of the entire mouse genome to locate novel therapeutic entry points for drug development. In combination with the information provided by the mouse model itself, Ingenium rapidly locates the gene responsible for the phenotype by positional cloning and gathers extensive knowledge of the gene's function and how the alteration influences the biological mechanisms that result in disease (1). The aim of this work was to identify novel genes involved in inflammatory bowel disease (IBD), to characterize their molecular functions and evaluate their therapeutic potential. A variant mouse line, MONTEZUMA, was identified which suffers from chronic diarrhea and impaired growth. Histologic analysis demonstrated morphological goblet cell abnormalities and inflammatory infiltration of intestinal epithelia. Deficient mucus production precedes inflammatory responses, suggesting that a primary defect in mucosal barrier function can lead to IBD-like symptoms in mice. The causative mutation was identified by positional cloning and found to affect a previously uncharacterized gene expressed in mucosal tissues. Detailed analysis of the phenotype and molecular characterization of the mutated gene will be presented. We identified a mutation in a novel gene leading to IBD-like symptoms in mice. The pathomechanism involves deficient mucus production by the intestinal epithelium, which precedes mucosal inflammation. We are currently investigating the therapeutic use of the protein in inflammatory bowel disease and the diagnostic value for prediction of disease predisposition in humans. References: (1) Russ A, Stumm G, Augustin M, Sedlmeier R, Wattler S, Nehls M. Random mutagenesis in the mouse as a tool in drug discovery. *Drug Discov Today*. 2002 Dec 1;7(23):1175-83. Review.

TGF- receptor mutations in early-onset pulmonary arterial hypertension. *R.E. Harrison¹, R.M.F. Berger², S.G. Haworth³, R.M. Tulloh⁴, C.J. Mache⁵, N.W. Morrell⁶, R.C. Trembath¹.* 1) Division of Medical Genetics, University of Leicester, Leicester, United Kingdom; 2) University Hospital Groningen, Netherlands; 3) Institute of Child Health, UCL, London, UK; 4) Guys and St Thomas Hospital, London, UK; 5) Medical University Graz, Austria; 6) Addenbrookes Hospital, University of Cambridge, UK.

Pulmonary arterial hypertension (PAH) may develop in association with systemic or cardio-pulmonary disease but many cases appear idiopathic (IPAH). Low penetrance mutations of *BMPR2* and *ALK-1*, encoding receptors in the TGF- signalling pathway, underlie the development of IPAH in adult life. PAH may also present in childhood, when it can be related to congenital heart defects (CHD) but is frequently idiopathic. We investigated the role of TGF- signalling in the aetiology of early-onset PAH.

Eighteen children presenting with PAH before the age of 6 years were identified; 14 with a diagnosis of IPAH and 4 with PAH and congenital heart defects. Mutation analysis of genes encoding receptors of the TGF- family revealed heterozygous mutations in 22% (4/18) patients.

A novel missense mutation in the NANDOR box region of *ALK-1* (1/18, 5.5%), and a novel branch site mutation of *endoglin* (1/18, 5.5%) were identified in children presenting with IPAH, highlighting the potential for hereditary haemorrhagic telangiectasia to present with early onset PAH.

Partial gene deletion of *BMPR2* was seen in two patients (2/18, 11%); one diagnosed with IPAH, and one child with PAH and a ventricular septal defect. Similar cardiac defects are seen in animal models of defective BMP signalling raising the possibility that *BMPR2* mutations may lead to congenital heart defects in humans as well as a predisposition to PAH.

Although the aetiology of early onset PAH is heterogeneous, disruption of TGF- signalling plays a vital role, with diverse genetic defects identified in this cohort.

PITX2 gain-of-function in Axenfeld-Rieger eye model. *T. Hjalt*¹, *J. Holmberg*¹, *C.-Y. Liu*². 1) Dept Cell & Molecular Biology, Lund University, Lund, Sweden; 2) Bascom Palmer Eye Institute Miami, FL.

The Axenfeld-Rieger syndrome is autosomal dominant and affects the development of eyes, teeth, and gut. The eye manifestations include cloudy corneas, tearing, glaucoma, iris hypoplasia, irido-corneal adhesion, and occasionally megalocornea. Mutations have been found in the genes for transcription factors PITX2 and FOXC1. Mice homozygous for disruption of PITX2 are embryonic lethal from heart malformation. These mice also have hypertrophic corneas. Most mutations found in humans are defective in either DNA binding or reporter gene transactivation in cell culture. One mutation previously studied exhibited increased transactivation. Another has been shown to be dominant negative. Here we have made a transgenic mouse model, overexpressing normal human PITX2A in the cornea. These mice present with cloudy corneas, tearing, irido-corneal adhesions, mild to severe corneal hypertrophy and severe progressive retinal degradation. The corneal collagen superstructure is disrupted. Proposed PITX2 target gene procollagen lysyl hydroxylase 2 (Plod2) is down-regulated by whole eye RTPCR. The proposed cardiac PITX2 target gene cyclin D2 is not affected. This model provides in vivo evidence for a gain-of-function pathology for PITX2 causing Axenfeld-Rieger-like eye symptoms. In addition to the eye phenotypes, there is a random left-right forelimb malformation. The radius is curved over the ulna, locking the elbow in a pronation-extension position.

Isolation and characterization of immortalized renal cells from Pkd2 knockout mice. *J. Horst, M.P. Manitz, M. Polkehn, B. Dworniczak, P. Pennekamp.* Institut fuer Humangenetik, Universitaetsklinikum Muenster, Muenster, Germany.

Autosomal dominant polycystic kidney disease (ADPKD) is a frequent hereditary disorder, mainly characterized by the formation of multiple renal tubular cysts, leading to endstage renal failure. ADPKD is caused by mutations in the PKD1 and PKD2 genes, encoding the transmembrane proteins polycystin-1 (PC1) and polycystin-2 (PC2), respectively. In mouse knock-out models, homozygous impairment of the Pkd2 gene results in prenatal lethality. Because the death of these animals at the embryonic stage prevents a detailed functional analysis of PC2, we have isolated renal epithelial cells from Pkd2-deficient embryos. To establish immortalized Pkd2^{-/-} renal epithelial cell lines, heterozygous Pkd2^{+/-} mice were crossed with ImmortoMouse which is transgenic for the temperature sensitive SV40 large T antigen (H-2Kb-tsA58). We obtained Pkd2^{+/-} Immorto⁺ animals which were intercrossed to generate embryos with the various genotypes. To establish epithelial cell lines, embryonic kidneys (E16.5) were minced and digested with collagenase and epithelial cells were enriched with DBA-coated Dynabeads. Cells were seeded on VPM coated culture dishes and cultured under standard conditions described for kidney epithelial cells. Immunohistochemistry staining, FACS analysis and real-time RT-PCR were employed to study the expression of epithelial marker molecules. Cells isolated from control and PC2-deficient embryonic kidneys in culture displayed an epithelial phenotype. When seeded on collagen and cultured under non-permissive conditions (39°C, no Interferon), T-Antigen expression declined after 2 days of culture. All cells expressed lectins Dolichos biflorus agglutinin (DBA) and Phaseolus vulgaris (PHA-E). The adherens junction protein E-cadherin and its associated catenins, acetylated tubulin, and other marker molecules such as aquaporin-1 (AQP1), Ca²⁺-dependent Cl-channel 1 (ClCa-1), zonula occludens protein-1 (ZO-1) and epidermal growth factor receptor (EGF-R) have been detected in Pkd2^{-/-} Immorto⁺ and control cells. These immortalized renal epithelial cells from Pkd2 knock-out mouse embryos will allow the study of the role of polycystin-2 in signalling transduction pathways.

Bi-allelic inheritance and absence of somatic mutation in PPH provide evidence against a tumour suppressor role for BMPR2. *V. James¹, R.D. Machado¹, M.A. Aldred¹, M. Southwood², N.W. Morrell², R.C. Trembath¹.* 1) Division of Medical Genetics, University of Leicester, Leicester, Leicestershire, United Kingdom; 2) Department of Medicine, University of Cambridge, UK.

Idiopathic pulmonary arterial hypertension (IPAH) is a lethal disorder of the pulmonary vasculature typified by dysregulated endothelial cell proliferation and the formation of characteristic plexiform vascular lesions. Inheritance of familial PAH is complex, being autosomal dominant with incomplete penetrance and a significant female sex bias. Heterozygous germline mutations have been identified in bone morphogenetic receptor type II (BMPR2), a member of the transforming growth factor-beta (TGF-) signaling superfamily. When complexed with a type I receptor, BMPR-II relays intracellular signals by phosphorylating a series of cytoplasmic mediators, of which the Smad proteins are the most extensively characterized. Somatic mutations in genes encoding TGF- signaling molecules have been implicated in cancer progression (e.g. SMAD4 in juvenile polyposis, and TGFRII in gastrointestinal lesions). We describe a patient with biallelic pathogenic mutations of BMPR2. Biochemical studies revealed each variant to have distinct effects on downstream signaling yet cumulatively are permissive of residual Smad activity. The patient developed PAH at a stage (age 20 yrs) comparable to heterozygous carriers of mutant BMPR2 alleles. We next questioned if somatic mutations were evident in progressive pulmonary arterial disease. We used laser capture microdissection to collect plexiform lesions from lung explant tissue of 7 familial (three with identified germline mutations) and 2 sporadic patients, to investigate loss of heterozygosity (LOH) of BMPR2 and genomic instability, through analysis of 5 microsatellite markers, of key cell growth or apoptotic genes. We observed neither LOH nor microsatellite instability in any of the nine patient samples. Taken together these studies provide no support for multiple mutational events at the BMPR2 locus or acquired genome instability as significant factors in the progression of familial PPH.

Malcavernin, the cerebral cavernous malformations 2 protein, is preferentially expressed in neurons: Implications for CCM pathogenesis. *C.L. Liquori, T.L. Squire, N.W. Plummer, J.S. Zawistowski, H. Matsunami, D.A. Marchuk.* Dept Mol Genet and Microbiol, Duke University, Durham, NC.

Cerebral cavernous malformations (CCMs) are vascular abnormalities of the brain that can result in a variety of neurological disabilities, including stroke and seizures. Three known loci CCM1, CCM2, and CCM3 - are responsible for familial CCM. The CCM1 protein is KRIT1. The CCM2 protein is malcavernin. The CCM3 protein remains unknown. Conflicting data on the expression pattern of KRIT1-in situ analysis shows neuronal expression while immunostaining shows endothelial expression-has raised a fundamental question. Is the dysregulation of vascular morphogenesis leading to CCM formation a result of an intrinsic defect within the vasculature itself or a response to a defect in the surrounding brain tissue? Our identification of the CCM2 protein and preliminary evidence that malcavernin binds KRIT1 provide an independent means to better understand CCM pathogenesis and to resolve the discrepancy in the KRIT1 expression pattern. We examined expression of CCM2 using -gal histochemistry on CCM2 knockout (+/-) mice as well as in situ analysis and immunostaining of wildtype mice. LacZ staining revealed that CCM2 was present in both the cerebral cortex and cerebellum of adult brain. The in situ analysis also showed widespread expression in adult brain, with the strongest expression in Purkinje cells, hippocampus, caudate putamen, olfactory tubercle, and third and fourth ventricles. Very similar patterns were observed with in situ analysis of Krit1 and Icap1, a binding partner of KRIT1. The CCM2 in situ expression pattern closely resembled the pattern of the neuronal cell marker NSE. Immunostaining with a CCM2 antibody showed the strongest expression within the Purkinje cells. Again, the CCM2 pattern resembled that of another neuronal marker NF200, but it did not resemble the pattern of either the glial cell marker GFAP or the endothelial marker VWF. The CCM2 protein is expressed in neurons and not in the brain vasculature. Taken together, our data indicate that CCM lesions develop within the brain vasculature in response to altered signalling from the surrounding neuronal tissue.

Novel heterozygous compound of the CYP1B1 gene in primary congenital glaucoma. *O. Messina*¹, *C. Chima*², *L. Gonzalez*², *S. Kofman*², *M. Rivera*², *I. Babayan*¹, *S. Cuevas*². 1) Oftalmologia, Hospital General de Mexico, Mexico D.F., Mexico; 2) Genetica, Hospital General de Mexico, Mexico D.F., Mexico.

Primary congenital glaucoma (PCG) is a disease with an incidence that ranges from 1 in 1,1250 to 1 in 22,000 births in several populations. PCG seems to have an autosomal recessive mode of inheritance. PCG has been linked to homozygous mutations in the CYP1B1 gene that is located on chromosome 2p22-p21. The CYP1B1 gene contains 3 exons with an ORF of 1,629 bp starting in the second exon, and harbors nine TCDD-responsive (dioxin-responsive) enhancer core binding motifs. The CYP1B1 gene codifies for cytochrome P4501B1, a member of a subfamily of cytochrome P450. In the present study, we describe a family with PCG due to defective heterozygous compound of the CYP1B1 gene. This family also harbored a variant of the cytochrome P4501B1 previously reported in other population causing PCG. This mutation corresponded to a 13 bp deletion in exon 3 and a 1 bp deletion in exon 2 in both alleles of the CYP1B1 gene. More than a non-equal recombination event, we consider that this heterozygous compound was due to consanguinity of the region.

Kinetic alterations due to a missense mutation in the Na,K-ATPase alpha 2 subunit cause Familial Hemiplegic Migraine type 2. L. Segall¹, R. Scanzano¹, M.A. Kaunisto², M. Wessman², A. Palotie³, J.J. Gargus⁴, R. Blostein¹. 1) Biochemistry and Medicine, McGill University, Montreal, Quebec, Canada; 2) Biomedicum Helsinki and Clinical Chemistry, University of Helsinki, Finland; 3) Finnish Genome Center, University of Helsinki, Finland and Pathology and Human Genetics, University of California, Los Angeles, CA; 4) Human Genetics, Pediatrics, Physiology & Biophysics, University of California, Irvine, CA.

Several missense mutations in the Na,K-ATPase alpha 2 subunit gene (*ATP1A2*), have been identified in familial hemiplegic migraine with aura (FHM2). Loss-of-function and haploinsufficiency were the suggested mechanism of disease based on 2 mutants functionally unable to support *in vitro* cell growth. However a myriad of critical physiological processes rely upon normal pump function. This paper reports that the growth-failure phenotype is not shared by several other FHM2 alleles, and describes a kinetic analysis of mutant T345A, identified in a detailed genetic analysis of a large Finnish family (Kaunisto et al., *Neurogenetics*, 5: 141-46, 2004), that reveals a mutant-specific alteration in pump function. Introduction of T345A into the conserved rat alpha 2 enzyme does not alter cell growth or catalytic turnover but causes a decrease in apparent K⁺ affinity. In view of the location of T345 in the cytoplasmic stalk domain adjacent to transmembrane segment 4, the increase (2-fold) in K_{0.5(K)} is probably due to T345A replacement altering K⁺ binding/occlusion. Faster K⁺ deocclusion of the mutant via the E2(K) + ATP → E1ATP + K⁺ partial reaction is evidenced in (i) marked (300%) increase in K⁺-stimulation of Na-ATPase at micromolar ATP together with (ii) a 4-fold decrease in K_{ATP} and (iii) modest (3-fold) increase in I₅₀ for vanadate. We suggest that the decreased apparent K⁺ affinity is the basis for a reduced rate of extracellular K⁺ removal, delaying the recovery phase of nerve impulse transmission in the CNS, and thereby, the clinical picture of FHM2. This is the first demonstration of a mutation which leads to a disease resulting from a kinetically altered but fully functional Na,K-ATPase, refining the molecular mechanism of pathogenesis in FHM.

The incidence of small deletions of the HFE gene causing iron overload in the population of Ontario. *S.A.M. Taylor, M. Wing, L. Weiler, S. Windsor, A. Lyng, H.E. Feilotter, D.P. Lillicrap.* Pathology and Molecular Medicine, Queen's University and Kingston General Hospital, Kingston, ON, Canada.

There are two common mutations of the HFE gene, C282Y and H63D associated with hemochromatosis and iron overload. Rarely, other point mutations of the HFE gene causing amino acid substitutions and splicing abnormalities have been observed. The incidence of deletions of the HFE gene is unknown. At our centre the two common mutations are screened for in individuals with a clinical or family history of hemochromatosis or iron overload using PCR and diagnostic restriction enzyme digestion of exons 2 and 4 of the HFE gene. In 3 of 9800 cases analyzed we have observed additional (heteroduplex) bands after electrophoresis. Sequencing of the relevant exon revealed the presence of a small deletion in each case. The first is a novel 2 bp deletion of exon 4 (c.del640_641) observed in a male diagnosed with hemochromatosis and who is also a compound heterozygote for the C282Y mutation. The deletion is a frameshift mutation and is expected to result in a truncated form of the HFE protein. The second case occurred in a male of Japanese descent who presented with increased ferritin levels. He was found to have a novel 3 bp deletion of exon 4 (c.del691_693), which removes residue Y231. The effect of this mutation on the function of the HFE protein is unclear, however it is located in the alpha3 domain which is involved in beta2 microglobulin binding and it is conserved amongst other human and mammalian HFE and MHC Class I proteins. Iron overload in the Japanese population that is due to mutations of the HFE gene is rare and mutations other than the two common mutations have not been reported. From the sequence of the HFE gene both deletions are likely to have been the result of DNA strand slippage. These two unreported cases in addition to a 22 bp deletion of the HFE gene that was previously reported by us (Clinical Genetics 63: 163) would put the incidence of small deletions of the HFE gene in our population at 1 in 3270. This is a conservative estimate as only a portion of the HFE gene has been analyzed in each individual tested and the finding of heteroduplex bands was incidental.

Fibrinogen gene mutations: from rare bleeding disorders to common quality control pathways of protein secretion. *D. Vu, M. Neerman-Arbez.* Genetic Medicine & Development, University Medical Centre, Geneva, Switzerland.

Congenital afibrinogenemia is a rare bleeding disorder characterized by the complete absence of fibrinogen in circulation. Fibrinogen, synthesized in hepatocytes, is a hexamer composed of 2 sets of 3 homologous polypeptides i.e. A-, B-, and γ -chains, each chain encoded by a distinct gene, FGA, FGB, and FGG on chromosome 4q28-31. All afibrinogenemia mutations described so far are localized in the fibrinogen locus, the great majority in FGA. All are null apart from four missense mutations in FGB: L383R, G430D, G444S and W467G. The missense mutations lie in the C-terminal portion of the B-chain, which is highly conserved amongst vertebrates. In addition, two nonsense mutations in this same region were recently described: W467X and W470X. Expression studies in transfected cells performed for four of these FGB mutations suggested that an intact B C-terminal domain was necessary for hexamer secretion into the media but not intracellular hexamer assembly. Interestingly, this property of the fibrinogen B-chain differs from that of the γ -chain C-terminus which has been shown to be necessary for fibrinogen assembly. In this study, expression of serial deletions demonstrated that in the B C-terminal region, only the 6 last residues are dispensable for secretion, suggesting that an intact last beta strand is necessary for maintaining the tri-dimensional structure of the B D domain which in turn seems to be critical for fibrinogen secretion. Immunofluorescence co-localisation data demonstrated that a secretion-impaired fibrinogen FGB mutant (R485X deleted of only seven residues) is retained in the endoplasmic reticulum and is not transported to the Golgi apparatus. Co-immunoprecipitation experiments are ongoing in order to determine if other proteins e.g. molecular chaperones are involved in this quality control of fibrinogen secretion. The data should also allow us to gain insight into retention and degradation mechanisms of other mutant secreted proteins.

A third locus of Limb Girdle Muscular Dystrophy (LGMD) among Arabs in the north of Israel: LGMD2C in a large consanguineous Bedouin family. *Y. Goldberg*¹, *T. Harel*², *E. Chervinsky*¹, *R. Ofir*², *O.S. Birk*², *S. Shalev*^{1,3}. 1) Genetic Institute, HaEmek Medical Center, Afula; 2) Genetics Institute, Soroka Medical Center, Ben-Gurion University, Beer Sheva, Israel; 3) Rappaport School of Medicine, Technion, Haifa.

LGMDs represent a heterogeneous group of diseases characterized mainly by muscle wasting of the lower and upper limbs, with a wide range of clinical severity. To date, 10 genes associated with the autosomal recessive forms of LGMD have been identified.

We describe five members of a large consanguineous Bedouin tribe in the north of Israel, affected with a Duchene like form of LGMD, inherited in an autosomal recessive pattern. A genome-wide screen followed by fine-mapping revealed linkage to a region on chromosome 13 harboring the gamma-sarcoglycan gene (SGCG), associated with LGMD2C. A 525delT mutation was found in a homozygous state in all affected individuals. This mutation is common mainly in north Africa. Interestingly, our patients carry the mutation on a different haplotype background than the one associated with the mutation in the north African population. LGMD2C is the third type of LGMD detected among the Arab population in Israel. Two other forms have been previously described in Arab families: LGMD2I and LGMD2B. It is noteworthy that the 3 affected families belong to the same ethnic group and reside in great proximity in the north of the country. We searched for co-existence of a compound genotype, of mutations in both the SGC and FKRP genes (triallelic inheritance), as a possible explanation for the intra-familial phenotypic variability of LGMD2I; however, none of our patients inherited mutations in both genes.

As the clinical features of these three pathologies are different, diagnosis can be guided by the origin of the family, and established by clinical assessment followed by molecular testing. Besides the scientific significance of these findings, they might assist in the diagnosis of LGMD in middle-eastern populations in general, and in affected patients of Bedouin origin in particular.

Overexpression of Pitx1 gene activates muscle atrophy pathways: a pathological model of FSHD. *L. Li¹, Y.-W. Chen^{1, 2}*. 1) Center for Genetic Medicine Research, Children's National Medical Center, Washington, DC; 2) Department of Pediatrics, George Washington University, Washington, DC.

Muscle atrophy and weakness are the most common presenting manifestations of facioscapulohumeral muscular dystrophy (FSHD). We previously showed that paired-like homeodomain transcription factor 1 (Pitx1) was specifically up-regulated in FSHD patients and hypothesized that up-regulation of Pitx1 contributed to the pathological changes in FSHD. To identify the molecular targets of Pitx1, we expression profiled mouse triceps over-expressing Pitx1 gene using Affymetrix Mouse Genome 430A 2.0 microarrays. In addition, we investigated the effect of Pitx1 on myocyte proliferation and differentiation using C2C12 cells transfected with Pitx1 gene. The control samples were transfected with insertless vector. At day 3 after gene delivery by electroporation, immunofluorescence staining showed that the overexpressed Pitx1 protein was localized in the myonuclei. By comparing the profiles of Pitx1 overexpressing samples to controls collected at day 3 after gene delivery, we identified 22 and 15 known genes significantly up- and down-regulated in Pitx1 overexpressing samples, respectively (p<0.01). Five of the 22 genes were members of ubiquitin-proteasome pathways, including atrogin-1/MAFbx which has been reported strongly induced in skeletal muscle atrophy. The up-regulation of atrogin-1 was confirmed by quantitative RT-PCR (4.6 fold) (p<0.05, n=5). In addition, 4 enzymes responded to oxidative stress, including glutathione peroxidase 3, were significantly increased. At 7 days after gene delivery, we identified NADH staining-positive atrophic myofibers in the Pitx1 overexpressing muscles. Overexpression of Pitx1 in the C2C12 cells showed that the protein inhibited cell proliferation and induced early cell differentiation. In conclusion, our in vivo and in vitro data suggested that up-regulation of Pitx1 activated molecular pathways involved in muscle atrophy and disturbed cell proliferation and differentiation of myocytes, which might contribute to the pathophysiology of FSHD.

A novel mutation in the EDAR gene is responsible for anhidrotic ectodermal dysplasia in two Moroccan families.

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Hypohidrotic (HED) and anhidrotic ectodermal dysplasia in human (AED) and their mouse analogues tabby, downless, and crinkled (Ta/dl/cr) cause ectodermal defects of teeth, hair and sweat glands. The mutated genes, which produce a similar phenotype encode proteins of the tumor necrosis factor (TNF) signaling pathway: ectodysplasin (EDA-A1), a TNF ligand, its receptor (EDAR) and the intracellular adapter protein (EDARADD). The ectodysplasin-EDAR signaling pathway is involved in skin appendage morphogenesis. EDAR activates the NF-kB pathway, the activity of which is defective both in severe forms of anhidrotic ectodermal dysplasia and in downless mice. Hitherto few mutations in the autosomal genes EDAR and EDARADD have been reported. Here, we report the identification of a novel missense homozygous mutation (T59G) in the EDAR gene changing a Leucine into Proline at codon 20. This mutation was found in two unrelated Moroccan families with autosomal recessive ectodermal dysplasia, suggesting a founder effect. The mutation was not found in a series of 100 chromosomes matched for the ethnic origin. This mutation occurred in the signal peptide of EDAR and possibly affects the downstream signaling-transduction complex. Our study support the view that EDAR gene is both genetically and fonctionnaly heterogeneous with autosomal dominant and recessive modes of inheritance. Ongoing studies in additional Moroccan families, aimed to the goal of understanding how this mutation alters NF-kB activation, are currently in process.+ This work is supported by "Accord INSERM-CNCPRST".

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Congenital glaucoma: *CYP1B1* mutations in Israeli Bedouin Kindreds. *U. Bar Yosef, J. Levy, O. Agamy, K. Elbedour, R. Ofir, R. Carmi, O.S. Birk.* Laboratory of Human Molecular Genetics and the Genetics Institute, Soroka University Medical Center, Ben-Gurion University, Beer-Sheva, Israel.

Primary congenital glaucoma is an autosomal recessive disorder presenting from birth to 3 years of age. In the absence of treatment, the high intra-ocular pressure leads to ocular enlargement (buphthalmos), corneal edema and progressive optic nerve cupping culminating in progressive loss of vision and ultimate blindness. Often, clinical diagnosis is made only after irreversible damage to the optic nerve has already occurred hence the importance of early diagnosis. Most cases of congenital glaucoma result from mutations in the *CYP1B1* gene, a member of the cytochrome p450 family. We now show that in 9 of 11 non-related affected consanguineous Israeli Bedouin families, the congenital glaucoma was associated with homozygosity of *CYP1B1* mutations. As in Saudi Arabian families, the 3987G>A *CYP1B1* mutation accounted for ~50% of cases. A novel *CYP1B1* mutation, 8405G>A, was found in two unrelated families. In two consanguineous families there was no evidence of homozygosity or mutations in either *CYP1B1* or in two other candidate genes, *ARNT* and *AhR*. Thus, our data suggest that other, yet unraveled gene(s) take part in the causation of the phenotype.

Screening for mutations of the CDCrel-1 and Pael-receptor genes in Parkinsons disease patients. *P. Bauer¹, A. Bob¹, I.S. Mäcke-Jentsch¹, D. Berg^{1,4}, D. Voitalla², T. Müller², K. Berger³, R. Krüger⁴, O. Riess¹.* 1) Department of Medical Genetics, University of Tübingen, Tübingen, Germany; 2) Department of Neurology, Ruhr-University Bochum, Bochum, Germany; 3) Institute of Epidemiology and Social Medicine, University of Münster, Münster, Germany; 4) Hertie Institute of Clinical Brain Research, University of Tübingen, Tübingen, Germany.

Mutations in Parkin have been implicated in autosomal recessive juvenile Parkinsonism (AR-JP). CDCrel-1, a synaptic vesicle-associated protein, is ubiquitinated by Parkin, which promotes degradation of CDCrel-1. Likewise a putative G protein-coupled transmembrane polypeptide, named Parkin-associated endothelin receptor-like (Pael-R), has been characterized as interactor with Parkin. This receptor accumulates in AR-JP brains. In order to analyze whether both genes are involved in familial Parkinsons disease, we performed a screening for mutations by means of denaturing High Pressure Liquid Chromatography (dHPLC). For both genes the complete open reading frame has been analyzed for heteroduplicates in pooled PCR amplicons, indicating heterozygous sequence variants. Overall, we analyzed 293 Parkinsons disease (PD) samples for both genes. For CDCrel-1 one aberrant elution profile could be detected in exon 7. Sequencing revealed a heterozygous silent nucleotide exchange (c.552C>T; p.I148I) in one PD patient. The latter has not been observed in more than 250 control alleles. For Pael-R only three common exonic non-synonymous sequence variants (c.48T>C, p.L16L; c.1047C>T, p.T349T; c.1329G>C, p.L443L) have been detected. Frequencies of these polymorphisms did not differ significantly between the PD patient group and a control group (242 control samples). In conclusion we did not find potential disease causing mutations neither in the CDCrel-1-gene nor in the Pael-R-gene in PD patients. Moreover, the comparison of allele frequencies for three common polymorphisms in these genes between PD patients and controls did not argue for an involvement of these functionally relevant genes in familial parkinsonism.

Lipoid proteinosis: Clinical heterogeneity in an extended Bedouin family with a novel splice-site mutation in the ECM1 gene. *R. Birnbaum, A. Zvulunov, L. Fuchs, R. Ofir, A.D. Cohen, K. Elbedour, O. Agami, O.S. Birk.* human genetics laboratory, Ben Gurion University of the Negev, Beer Sheva, Israel.

Lipoid Proteinosis (Urbach-Wiethe disease, MIM #247100) is an autosomal recessive disease that invariably affects the skin and mucous membranes and, occasionally, the central nervous system. The disease is caused by loss of function mutations in the extracellular matrix protein (ECM1) gene, coding for a secreted glycoprotein with 3 isoforms (ECM1a,b,c). So far, 18 different mutations in the ECM1 gene have been identified, mostly in exons 6 and 7, and all within the coding sequence. We describe a consanguineous extended Israeli Bedouin family with 5 individuals affected with the disease; all 5 were homozygous for a novel splice-site mutation in the intron 3' flank of exon 8 (truncating the C termini of the ECM1a, ECM1b and ECM1c isoforms). Although all the affected individuals shared the same mutation, there were pronounced differences in their clinical manifestations, proving clear phenotypic variability in lipoid proteinosis. Based on the findings to date, we suggest that the lipoid proteinosis phenotype might be at least in part the consequence of accumulation of mutant ECM1 protein, and not necessarily caused by ECM1 malfunction. Furthermore, it is unlikely that defects in the ECM1b isoform, known to be associated with keratinocyte differentiation, is essential in the disease process.

Screening of CFTR gene rearrangements in Italian CF patients. *C. Bombieri*¹, *O. Raguénès*², *M.P. Audrézet*², *A. Bonizzato*³, *C. Castellani*³, *C. Férec*², *P.F. Pignatti*¹. 1) Sec Biol & Genetics, Dpt. Moth. Child & Biol-Genet, Univ Verona, Verona, Italy; 2) Inserm Unit 613, University Hospital, Brest, France; 3) Veneto Rgional CF Center, Verona, Italy.

Despite the extensive efforts over the past years, a significant percentage of CF alleles still remain to be identified in most populations. Only a few large deletions have been found so far in the CFTR gene. For this reason, it has been suggested that genomic rearrangements could account for a significant percentage of the unidentified disease alleles. These mutations, if present at the heterozygous state, elude the conventional PCR-based screening methodologies commonly used, including direct sequencing, and denaturing high performance liquid chromatography (DHPLC), leading to the underestimation of large genomic rearrangements involving the CFTR gene. The first systematic screening for large rearrangements of the 27 exons of the CFTR gene was recently performed, using quantitative multiplex PCR amplification of short fluorescent fragments (QMPSF), in French CF patients (Hum Mut 23:343,2004). We used this method to analyse a well characterised cohort of 25 Italian CF patients: all had classical CF and carried at least one unidentified allele after complete CFTR gene screening by DGGE or DHPLC analysis. Three rearrangements were identified in 6/26 of the previously unidentified Italian alleles (21%): an ins/del mutation involving exon 1 (c.4-IVS1+69del119bpins299bp), previously described in French CF patients, was found in 2 alleles; a deletion spanning exons 17a to 18 (3120+1Kbdel8.6Kb), previously described in Palestinian CF patients, was found in 3 alleles. Moreover, a deletion of exon 11 was found in 1 allele: at present the characterization of this mutation is going on. These results are in agreement with the data obtained in the French cohort (Hum Mut 23:343,2004).

Germline mosaicism for *de novo* mutations of the calcium-sensing receptor (*CASR*) gene: implications for genetic diagnosis of hypoparathyroidism. R.A. Booth^{1,2}, B.Y.L. Wong¹, G.N. Hendy³, D.E.C. Cole^{1,2,4,5}. 1) Dept of Clinical Pathology, Sunnybrook & Women's College Health Sciences Centre, Toronto ON; 2) Dept of Laboratory Medicine & Pathobiology, Univ of Toronto, Toronto ON; 3) Depts of Medicine, Physiology & Human Genetics, McGill Univ, Montreal QC; 4) Dept of Medicine, Univ of Toronto, Toronto ON; 5) Dept of Genetics, Hosp for Sick Children, Toronto ON, Canada.

Patients with unexplained isolated hypoparathyroidism may carry *de novo* activating mutations of the *CASR* gene, associated with autosomal dominant hypocalcemia (ADH) and hypercalciuria. Using routine PCR and restriction digestion of genomic DNA from peripheral blood, we identified one copy of a novel F788L mutation in two affected siblings, but none in either parent. The mother was subsequently found to be a mosaic for the mutation, based on (1) denaturing HPLC (dHPLC) screening, and (2) sequence analysis of multiple clones derived from the involved PCR amplicon (*J Clin Endocrinol Metab* 2003;**88**:3674).

We therefore undertook screening of five additional families, each with a single affected offspring bearing a mutation considered *de novo* by conventional testing. In three families, inactivating mutations (F42S, R185Q, C582Y) were associated with familial hypocalciuric hypercalcemia (FHH), while the other two carried activating mutations (E228K, N802I) responsible for ADH. Screening by dHPLC was negative in 4 families but a small shift in the profile of the E4-1 amplicon (the 5' end of exon 4) was noted in the mother of the child with an E228K mutation. Maternal mosaicism was confirmed by allele-specific oligonucleotide (ASO)-based amplification capable of detecting 2% heterozygosity. Peak heights of the sequencing profile (nt682GA) and dilution studies using ASO amplification suggested 10-20% heterozygosity in maternal blood. In summary, we have detected maternal mosaicism in 2 out of 6 families for whom clinical assessment and routine testing indicated a mutation arising *de novo* in the proband. Caution should be exercised in counseling such families for recurrence risks until a careful search for parental mosaicism is undertaken.

NIPBL mutations and genetic heterogeneity in Cornelia de Lange syndrome. *G. Borck¹, R. Redon², D. Sanlaville¹, M. Rio¹, M. Prieur¹, M. Vekemans¹, N.P. Carter², A. Munnich¹, L. Colleaux¹, V. Cormier-Daire¹.* 1) INSERM U393, Hopital Necker-Enfants Malades, Paris, France; 2) Wellcome Trust Sanger Institute, Genome Campus, Hinxton, UK.

Cornelia de Lange syndrome (CdLS) is characterized by facial dysmorphism, microcephaly, growth and mental retardation, and congenital anomalies including limb defects. Recently, mutations in the human NIPBL gene have been found in approximately 20% of CdLS cases.

We present a comprehensive clinical, cytogenetic, and molecular genetic study of 14 children with CdLS. The series included seven boys and seven girls ranging in age from 14 months to 17 years. All had typical dysmorphic features, pre- and postnatal growth retardation, mental retardation, and microcephaly. None had limb reduction defects. The study included a high-resolution chromosome analysis, a search for small chromosome imbalances using array-CGH at ~1 Mb resolution, and direct sequencing of the 46 NIPBL coding exons.

Cytogenetic or molecular genetic anomalies likely to underlie CdLS were found in 6/14 cases, including two chromosome imbalances and four NIPBL mutations. Chromosome analysis detected an isochromosome 18p in a girl with a mild CdLS phenotype. By array-CGH, a girl with severe manifestations of CdLS was found to have a submicroscopic de novo deletion of chromosome 1q. We detected four heterozygous de novo NIPBL mutations including one splice-site (IVS2+1 G>A), one frameshift (95delT), and one missense (N2236I) mutation and a deletion of three nucleotides (leading to N2218del). No mutations were found in the two children with a chromosome imbalance. We could exclude deletions encompassing the NIPBL locus by array-CGH as BAC clone RP11-7M4 gave a normal hybridisation signal in all cases.

We conclude that NIPBL mutations account for ~30% of CdLS cases in our series. There is allelic heterogeneity at the NIPBL locus in CdLS, as there is no recurrent mutation reported to date. The absence of NIPBL mutation in 70% of CdLS cases and the observation of two distinct cytogenetic anomalies is highly suggestive of genetic heterogeneity.

Mutation screening of *LRP5* in familial exudative vitreoretinopathy (FEVR) patients. *H.M. Bottomley¹, L.M. Downey¹, D.A. Mackey², J.E. Craig³, C.F. Inglehearn¹, C. Toomes¹.* 1) Molecular Medicine Unit, University of Leeds, Leeds, United Kingdom; 2) Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital, Melbourne, Australia; 3) Department of Ophthalmology, Flinders Medical Centre, Adelaide, Australia.

Familial exudative vitreoretinopathy (FEVR) is an autosomal dominant disorder which affects the development of the retinal vasculature. Using a positional candidate approach we recently identified mutations within the low-density-lipoprotein receptor-related protein 5 gene (*LRP5*) in FEVR families. In this study we screened *LRP5* in a panel of 60 FEVR patients to ascertain the type, frequency and location of mutations within this gene. We used intronic primers to amplify the 23 *LRP5* exons from genomic DNA and screened these using a combination of single strand conformational polymorphism/heteroduplex analysis (SSCP-HA) and direct sequencing. This work is still ongoing but to date we have identified more than ten mutations within *LRP5* including a splice site mutation (c4488+2tg), truncating mutations (K1374fsX1549, R1270fsX1438) and missense mutations (T173M, V667M, Y1168H and C1361G). None of these mutations were present in 200 control individuals. Molecular modeling of the missense changes within *LRP5* indicates that they are likely to either disrupt the correct folding of the protein or affect a proposed protein interacting domain. These results suggest that FEVR is the result of loss-of-function mutations in *LRP5* and that *LRP5* mutations are only responsible for 20% of FEVR.

Currarino Syndrome: Clinical and Genetic analysis of 25 cases. C. *CRETOLLE*¹, D. *SANLAVILLE*¹, J. *MARTINOVIC*², M. *ZERAH*³, C. *FEKETE*⁴. 1) GENETICS, Necker-Enfants Malades Hospital, PARIS, France, U393; 2) Foetopathology department; 3) Neurosurgery department; 4) Pediatric surgery department.

The Currarino triad is a variant of the caudal regression syndrome with typical sickle-shaped sacrum, anorectal malformation and presacral mass. It often displays a broad inter- and intrafamilial phenotypic variability. Some patients may have no symptoms. Mutations of the HLXB9 homeobox-gene, involved in sacral and anorectal development, have been recently identified as disease causing in the Currarino triad, with an autosomal dominant mode of inheritance. It has been mapped to the terminal end of human chromosome 7 (7q36). HLXB9 encodes the HB9 protein, a 403 amino acid transcription factor, that interacts with DNA through a specific homeodomain region of the protein. Here, we report the clinical and genetic retrospective and prospective study of 25 cases of Currarino syndrome (13 males and 12 females) including seven familial cases. All patients had a sacral malformation. An anorectal malformation was found in 23/25 cases. The presacral mass was an anterior meningocele in 5/25 cases, a teratoma in 4/25 cases, a neuroenteric cyst in 3/25 cases and a lipoma in 2/25 cases. There was no presacral mass in the remaining cases. All the 25 patients also had a tethered spinal cord. All these patients had a karyotype with FISH analysis at the HLXB9 locus and DNA sequencing of the HLXB9 coding sequence was performed. Five patients belonging to familial cases and two sporadic cases harboured a heterozygous point mutation of the coding sequence, that would predict a deleterious change in the protein (Ins C/nt53, Del A/nt587, Del G/nt711, Del GC/nt 732, Ins A/nt 783, L279F, S276X). All mutations involved the homeodomain, suggesting that amino acid changes in that region are relevant to the normal function of the protein. No mutation was found in other cases, and particularly in sporadic cases. Our data further support the involvement of HLXB9 in the Currarino triad, and its role in the development of the embryological tail bud development. Other genes probably account for cases with no mutation of the HLXB9 gene.

High heterogeneity of CFTR mutations in Iranian CF patients. *E. Elahi*^{1, 2}, *A. Khodadad*³, *M. Ronaghi*⁴, *Y. Thorstenson*⁴. 1) Dept Biological Sci, Tehran Univ, Tehran, Iran; 2) National Institute of Genetic Engineering and Biotechnology, Tehran, Iran; 3) Children's Hospital Medical Center, Tehran Univ, Tehran Iran; 4) Stanford Genome Technology Center, Stanford University, Palo Alto, CA.

All exons of the CFTR gene were sequenced in 60 Iranian CF patients to ascertain the mutation spectrum in the population. Twenty six putative disease causing mutations were identified, representing a very large mutation heterogeneity spectrum. Four of the mutations were novel: N185+1insA, N1821CA, N3713AC, N3811TG. F508 was the most frequent mutation, representing an expectedly low relative frequency of 15.8%. The six most common mutations together represented 43.3% of the mutated alleles. A haplotype analysis using ten intragenic polymorphisms identified 11 haplotypes and revealed that at least five mutations, including F508, were associated with more than one haplotype, indicating that these are rather old mutations in the population. A carrier frequency of 2.5 in 100 for CFTR mutations was estimated, suggesting that cystic fibrosis is a fairly common under-diagnosed disease in Iran. Eighteen putative non-disease causing polymorphisms were also identified, three of which are novel.

MECP2 gene deletions account for ~10% of Rett syndrome cases. *P. Fang*¹, *W. Jin*¹, *D.G. Glaze*³, *A. Percy*⁴, *H.Y. Zoghbi*^{2, 3}, *B.B. Roa*¹. 1) Baylor DNA diagnostic laboratory, Dept Molec Human Genetics, Baylor Col Medicine, Houston, TX; 2) Dept Molec Human Genetics, Baylor Col Medicine, Houston, TX; 3) Dept Pediatrics, Baylor Col Medicine, Houston, TX; 4) Dept Pediatrics, Univ.of Alabama, Birmingham, AL.

Rett syndrome is an X-linked neurodevelopmental disorder that affects ~1 in 10,000 females. Rett syndrome is caused by mutations in the MECP2 gene encoding the transcriptional repressor methyl CpG-binding protein 2. Diagnostic testing has mostly involved sequencing of exons 2 through 4 of the MECP2 coding region, which can identify mutations in approximately 80% of patients. MECP2 gene deletions have also been reported, although the overall frequency has not been well-defined. We developed a quantitative Southern analysis for large rearrangements in MECP2. Heterozygous deletions are detected by dosage differences of bands for exons 1 through 4. We performed Southern analysis on 153 patients who previously tested negative on MECP2 sequencing. This group included 36 patients with a thoroughly documented diagnosis of Rett syndrome, and 117 patients with varying amounts of clinical information provided by various referral sources. A total of 33 patients tested positive for MECP2 deletions which mostly involve exons 3 and 4; one patient had an insertion of ~100 bases in exon 4. Representative Southern data correlated with other quantitative methods like multiplex ligation probe amplification (MLPA) or real-time PCR. Analysis of our subset of well-characterized Rett patients indicate that MECP2 mutations identified by sequencing account for ~85% of cases, and MECP2 gene rearrangements comprised mostly of deletions account for ~10%. Thus, MECP2 deletions account for a substantial fraction of Rett syndrome patients. A combined strategy of sequencing and deletion analysis of a single gene, MECP2, can therefore provide a detection rate approaching 95% for Rett syndrome molecular genetic testing.

Sequencing of *IRF6* exons detects 67% and 90% of mutations in Van der Woude and Popliteal pterygium syndromes. R. Ferreira de Lima^{1,2}, S.A. Hoper¹, M. Johnson¹, S. Kondo¹, M. Fang¹, T.M. Zucchero¹, S. Daack-Hirsch¹, A.M. Mach¹, J.C. Murray¹, B.C. Schutte¹. 1) Pediatrics, The University of Iowa, Iowa City, IA; 2) Aconselhamento Genético, UNESP, Botucatu, Brazil.

Mutations in Interferon Regulatory Factor 6 (*IRF6*) cause Van der Woude (VWS) and Popliteal Pterygium syndromes (PPS). We completed sequencing on all exons in 129 unrelated VWS samples and 20 unrelated PPS samples and identified the following missense (M), protein truncation (PT), and splice site (S) mutations in our VWS and PPS (in parentheses) samples.

Exon	1	2	3	4	5	6	7	8	9	Total
M	0 (0)	0 (0)	9 (0)	13 (14)	0 (0)	1 (0)	17 (0)	5 (0)	1 (1)	46 (15)
PT	1 (0)	1 (0)	6 (0)	5 (0)	2 (0)	7 (1)	5 (0)	3 (1)	9 (1)	39 (3)
S	0 (0)	0 (0)	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)
Total	1 (0)	1 (0)	15 (0)	19 (14)	2 (0)	8 (1)	22 (0)	8 (1)	10 (2)	86 (18)

The 46 new VWS and 5 new PPS mutations place the detection rate for exon sequencing at 67% and 90%, respectively. The over-representation of missense mutations in the DNA binding (exons 3 and 4) and protein binding (exons 7 and 8) domains, indicate their functional importance. Previously we reported 2 large and 9 probable deletions in VWS cases. No mutations or deletions were detected in 32 VWS pedigrees. Analysis of the largest pedigree suggests linkage to *IRF6*. Although small, 8 pedigrees are consistent with autosomal dominant inheritance. Since little evidence exists to suggest genetic heterogeneity, we conclude that a significant number of VWS-causing mutations are located outside the exons of *IRF6*. We used comparative genomic analysis to identify potential regulatory elements and are sequencing them for mutations.

Twenty novel mutations revealed by DHPLC analysis of the neurofibromatosis type 1 (NF1) gene in unselected southern Italian NF1 patients. *A. Gabriele¹, M. Ruggieri^{2,3}, G. Peluso¹, T. Sprovieri¹, A. Patitucci¹, A. Magariello¹, M. Muglia¹, R. Mazzei¹, F. Conforti¹, S. Genovese², E. Ciancio², A. Quattrone⁴.* 1) ISN, CNR, Cosenza, Italy; 2) ISN, CNR, Catania, Italy; 3) Department of Paediatrics, University of Catania; 4) Institute of Neurology, University of Magna Graecia, Catanzaro.

The identification of mutations in the neurofibromatosis type 1 (NF1) gene has presented a considerable challenge because of the large size of the gene, the lack of significant mutational clustering, the diversity of the underlying pathological lesions and the presence of NF1 pseudogenes. Recently, denaturing high performance liquid chromatography (DHPLC) has been successfully applied to the mutational screening of NF1 yielding mutation detection rates of between 68% and 72.5%. To further testing its suitability in a routine diagnostic setting we evaluated prospectively, by means of DHPLC and DNA sequencing, the full coding region (60 exons) and splice junction of the NF1 gene in a panel of 85 consecutive, genetically uncharacterized, NF1 patients (43 familial and 42 sporadic cases) from Sicily and Calabria (southern Italy). Germ-line mutations were identified in 68 subjects (80%): 20 of these alterations were novel including two stop codons: c.3574G>T (E1192X) and c.4078C>T (Q1360X); five nucleotide substitutions: c.3327A>C (L1109F), c.3577T>A (F1193I), c.4180A>G (N1394D), c.4193T>A (V1398D), c.6364G>C (E2122Q); five small insertions: c.310_1insTAGCATAAACGATGCTGGTCCAGCA, c.1074_5insGAACCTGCTTTTT, c.4511_2insA, c.6488_9insA, c.6792_3insC ; six small deletions: c.4508delG, c.4625delA, c.5524delA, c.7017delT, c.7084_9delAACTCT, c.7365delT and two splice site mutations c.4269+1G>A, c.7806+1G>A. None of the novel mutations was detected in 100 control chromosomes from a group of healthy individuals from the same southern Italian regions. These novel mutations further contribute to the definition of the germ-line mutational spectrum of NF1. DHPLC confirms to be a rapid, efficient and accurate tool for NF1 mutational analysis. The mutation detection rate of 80% in the present study is the highest so far reported by means of DHPLC analysis.

Additional mutations in *SMAD4* in the combined Juvenile Polyposis-Hereditary Hemorrhagic Telangiectasia syndrome. C.J. Gallione¹, T. Leedom¹, M. Faughnan², T. Berk³, K.J. Henderson⁴, J. Beis⁵, M.D. Ludman⁵, R.I. White⁴, D.A. Marchuk¹. 1) Dept. of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC; 2) Dept. of Medicine, University of Toronto, Toronto, Canada; 3) Mount Sinai Hospital, Toronto, Canada; 4) Dept. of Diagnostic Radiology, Yale University School of Medicine, New Haven, CT; 5) Dept. of Pediatrics, Dalhousie University Faculty of Medicine, IWK Health Centre, Halifax, Canada.

Juvenile Polyposis (JP) and Hereditary Hemorrhagic Telangiectasia (HHT) are two uncommon autosomal dominant disorders characterized by distinct and essentially non-overlapping clinical features. JP, an inherited gastrointestinal malignancy predisposition, is caused by mutations in either *SMAD4* or *BMPRIA*. HHT is a vascular malformation disorder caused by mutations in either *Endoglin* or *ALK1*. All four of these genes encode proteins involved in the TGF-signaling pathway. We previously reported 7 families segregating both JP and HHT phenotypes that were all found to have mutations in the carboxy terminus of *SMAD4*. None of these had mutations in either *Endoglin* or *ALK1*. We have continued to collect samples from unrelated patients and families with this combined JP/HHT syndromic phenotype in order to validate these initial findings. We have performed sequence analysis of *Endoglin*, *ALK1*, *SMAD4*, and *BMPRIA* and have found three more cases where the affected patients have mutations in *SMAD4*. These three represent *de novo* cases bringing the total of documented *de novo* cases of this syndrome to 6 out of our total of 10 instances. This high incidence of *de novo* cases could have important implications for genetic screening and counseling of both patients and families.

Molecular analysis of the CRYG-D gene in a family with congenital lamellar cataract. *L. Gonzalez¹, O. Messina², S. Kofman¹, O. Sanchez¹, S. Cuevas¹.* 1) Genetica, Hospital General de Mexico, Mexico D.F., Mexico; 2) Oftalmologia, Hospital General de Mexico, Mexico D.F., Mexico.

Congenital or infantile cataract is one of the most common cause of blindness in childhood. It occurs in 1-6 cases per 10, 000 births and has multiple causes. Cataract may be present as the only ocular affliction or as a part of an inherited disorder. Most inherited cataracts, in which solely the lens is involved, harbor an autosomal dominant pattern. Lamellar cataract have also been called zonular, perinuclear or polymorphic. The degree of opacification is variable and visual acuity may be preserved or reduced. It occurs at the anterior and posterior Y sutures. CRYG genes encode the beta and gamma crystallins, the major lens proteins. The family of CRYG genes is a cluster of six closely related genes located on human chromosome 2q33-35. Previous studies in families with congenital lamellar cataract report mutations in the CRYG-D gene. In the present study, we analyzed the CRYG-D gene in a family that included 21 affected individuals in four generations with congenital lamellar cataract. The patients showed a different evolution of the symptoms and some of them harbored a high degree of myopia. DNA sequencing analysis of the peripheral blood showed no molecular defects, only polymorphic changes were found to be present. This result excludes the participation of the CRYG-D gene in congenital lamellar cataract in our family indicating the genetic heterogeneity of this type of cataract.

A Novel Mutation in SPG4 Gene Causes Autosomal Dominant Hereditary Spastic Paraplegia. *S.M. Grzeschik, A. Lewis, C.H. Wang.* Neurology, Stanford University, Stanford, CA.

Hereditary spastic paraplegia (HSP) is a heterogeneous group of neurodegenerative disorders with clinical features of progressive and bilateral spasticity of the lower limbs. The inheritance of HSP includes autosomal dominant (AD-HSP), autosomal recessive (AR-HSP) and X-linked (X-HSP) transmissions. Mutations leading to AD-HSP have been localized to chromosomes 14q, 2p, 15q, 8q and 12q. About 40 % of AD-HSP patients show mutations in the SPG4 gene on locus 2p. We report a novel mutation in the SPG4 gene that causes AD-HSP in a family with multiple affected individuals. Three affected individuals (the propositus - a three year old girl, her mother, and her 2 year old half-brother from the same mother but a different father) and two unaffected family members (the step-father of the patient, and her other half-brother) were evaluated in the clinic by two pediatric neurologists. All affected patients exhibited typical clinical features of HSP. We screened for mutations in SPG3, SPG4, and NIPA1 genes by complete sequencing of the coding regions in the index case. We discovered a missense mutation at nucleotide position 1289 (T-> C) in exon 8 of SPG4 that resulted in an amino acid change from lysine to asparagine. This mutation was found in all three symptomatic family members but in none of the two asymptomatic members. We also screened 56 unrelated control chromosomes and none of these contained this sequence variation. This novel mutation on the SPG4 gene is, therefore, considered disease-causing in this family. A previously reported mutation localized to the same codon at position 1288 (A->G) resulting in a change of amino acid from lysine to arginine also proved to be disease causing. The current finding supports the notion that the lysine residue at this position plays a crucial role in the SPG4 function.

Detection of CFTR Gene Mutations in Iranian Cystic Fibrosis Population. *M. H Darvishi¹, T. Hamzehloei¹, F. Bayat¹, M. Karimi¹, A. Khatami².* 1) Biotechnology Department, Pasteur institute of Iran, Tehran, Iran; 2) Emam khomini Hospital-Tehran-Iran.

Cystic Fibrosis is an autosomal disorder caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene, which may cause a common lethal disease. Since isolation of the gene more than 1000 mutations have been reported to the Cystic Fibrosis Analysis Consortium. The frequency of these mutations varies in different populations. Identification of mutations causing Cystic Fibrosis in the Iranian populations is essential for assessment of the molecular basis of CF in Iran and development of strategies for prenatal diagnosis and genetic counseling. In the present study, we report the mutations found in 33 Iranian CF families by heteroduplex analysis on MDE gel matrix. We found the following six mutations: F508, 2183AAG, 574delA, D110H, R334W, 2043delG, and the frequency of the mutations were 25.75%, 4.54%, 3.03%, 1.51%, 1.51% and 1.51% respectively. The frequency of major CF mutation (F508) was less in Iranian population than the worldwide spectrum and almost same as the Turkish population. This study could only detect 36% of disease-causing mutations in this population, that reflecting the high molecular heterogeneity of the Iranian population.

CTDP1 mutations in consanguineous families with typical or partial CCFDN. *T. Hamadouche*^{1,2}, *C. Lagier-Tourenne*³, *R. Bernard*⁴, *I. Boccaccio*², *H. Dolfuss*⁵, *N. Philip*⁴, *N. Levy*^{2,4}. 1) Laboratoire de Biologie Moleculaire, Institut Pasteur, Alger, Algeria; 2) INSERM U491, Faculte de Medecine, Marseille, France; 3) IGBMC, Strasbourg, France; 4) Genetique Medicale, Hopital d'Enfants, Marseille, France; 5) Federation de Genetique, CHU Strasbourg, France.

Congenital cataracts facial dysmorphism neuropathy (CCFDN) syndrome is a complex autosomal recessive disorder involving multiple systems and organs. Initially reported from the Roma Gypsy group originating from Bulgaria, CCFDN is characterized by developmental impairments affecting eyes and face, peripheral and central nervous system, growth and intellect. Recently, the disease causing gene was identified, CTDP1, encoding an essential component of the eucaryotic transcription machinery. So far, a unique founder homozygous mutation, g.IVS6+389CT, has been identified in CCFDN patients, resulting in a mechanism leading to aberrant splicing and causing partial loss of expression. In this study, we report molecular analysis of three consanguineous Gypsy families with either typical or partial CCFDN. The homozygous mutation was identified in families G and B. Indeed, although typical CCFDN phenotype was observed in patients from family G, in family B, a 5 years old girl mainly presented a demyelinating neuropathy with congenital cataract although developmental delay and facial dysmorphism were slight. In family L, initially diagnosed as non syndromic CMT4, the same mutation was found to be heterozygous in one patient. We are investigating other loci already known to segregate in Gypsies (NDRG1, HMSN-R) and transcriptional studies are in progress from cultured lymphocytes from CCFDN individuals of these families. However, despite the homozygous presence of the mutation in one affected patient (family B), the clinical phenotype is somewhat different, either suggesting that modifying factors are involved in the disease course or, more likely, that typical CCFDN, due to the severe evolution of this syndrome, might be misdiagnosed at lower ages. We thus propose CTDP1 as a candidate gene in patients affected with a combination of peripheral neuropathy and congenital cataract, even in absence of others developmental features.

Frequent Occurrence of DNA Rearrangements in the Cystic Fibrosis Transmembrane Conductance Regulator Gene. *F.M. Hantash, J.B. Redman, B. Anderson, C. Tubman, K. Starn, A. Buller, M. McGinniss, F. Quan, W. Sun, C.M. Strom.* Department of Molecular Genetics, Nichols Institute, Quest Diagnostics Inc., San Juan Capistrano, CA.

Of the >1000 CF mutations described thus far, almost all are point mutations or small base pair insertions or deletions. The frequency of the few single or multiple exonic rearrangements described may represent gross underestimation because DNA sequencing, the primary method of mutation detection, will not detect most single or multiple exon deletions or duplications. To detect such mutations, we developed a single tube Semi Quantitative Fluorescent PCR assay that amplifies fragments representing the promoter and all 27 CFTR exons and 3 internal controls from different chromosomes for signal normalization. Using this method, we analyzed DNA samples submitted for extensive CFTR sequencing and several samples with rearrangements were identified. A deletion encompassing the CFTR promoter, and exons 1 and 2 was detected in one sample, and the same mutation was detected in the maternal DNA. In another family, a deletion of the promoter and exon 1 was detected in three siblings. It is interesting to note that in both of these cases, the families were African-American, and a 3120+1G>A splice site mutation was identified on the other chromosome. These deletions have not been described before. In a third case, a Caucasian patient, a deletion of exons 17a, 17b and 18 was identified and the same mutation was detected in the paternal DNA. In four other cases, we identified the following deletions: exons 2 and 3; exons 4, 5 and 6a; exons 17a and 17b; and exons 22, 23 and 24. These mutations would remove parts of transmembrane domain 1, transmembrane domain 2, or the second nucleotide binding domain. In patients diagnosed with classic CF submitted for sequencing analysis, 22.6% (7/31) harbored rearrangements, accounting for 11% of CF chromosomes. The frequency of occurrence of rearrangements in classic CF patients when only one mutation is identified by DNA sequencing is 50% (7/14). It is possible that complex abnormalities may account for a significant proportion of CF chromosomes in the general population.

Connexin 26 (GJB2) gene mutations in different populations of Iran. *M. Hashemzadeh^{1,2}, D.D. Farhud¹, H. Hoghooghi Rad³, M. Dolati³, R. Sasanfar⁴, A. Hoseinipour⁴, A. Tolooi⁴, M. Ghadami⁴, M. Montazer Zohour¹, L. Andonian¹, H.R. Pourjafari⁵, M.A. Patton⁶.* 1) Dept. of Human Genetics, School of Public Health, Tehran University of Medical Sciences, Iran; 2) Dept. of Biochemistry and Genetics, Medical School, Shahrekord University of Medical Sciences, Iran; 3) Department of Genetics, School of Biology, Azad University, Tehran, Iran; 4) Dept. of Exceptional Children, Ministry of Education and Training, IR Iran; 5) Genetic Div., Medical School, Hamadan University of Medical Sciences, Iran; 6) Medical Genetics Unit, St Georges Hospital, Medical School, University of London, UK.

Autosomal recessive and sporadic non syndromic hearing loss (ARSNSHL) is the major form of hearing deafness. Mutations in the GJB2 gene encoding Connexin 26 (Cx26) protein are the main cause for ARSNSHL in many populations. To determine the role of the GJB2 mutations as a cause of deafness in different populations of Iran, 952 hearing impaired students and their deaf siblings from 781 families in 9 provinces of Iran (Tehran, Azarbayjan sharqi, Gilan, Khorasan, Sistan & Baluchestan, Hormozgan, Kordestan, Khuzestan and Golestan) were studied. The prevalence and type of the GJB2 gene mutations were investigated using nested PCR pre screening strategy and direct sequencing of the coding exon of the gene. Altogether 32 different genetic variants were detected. Cx26 mutations were found in 18% of the deaf families including T8M, 35delG, W24X, V27I+E114G, R32H, V37I, E47X, 167delT, W77X, 235delC, L90P, delE120, R127H, Y136X, R143W and R184P. We found Cx26 mutations in both alleles in 11.5% of deaf families. However, 35delG mutation was the most common GJB2 mutation accounting for 72.3% of the Cx26 mutations in populations studied. This study identified 9 novel variants H16R, E101E, K102Q, 327delG, 327delG+G109G, G130A, 431insC, R159H and G200R. Our data indicated a high rate of Cx26 mutations types and frequencies exists in different populations of Iran. The presented significance of Cx26 mutations in development of hearing loss advocates their usage as a basis of further prenatal genetic counseling and clinical guidelines.

Molecular analysis in Gitelman Syndrome: 43 novel mutations in a cohort of 93 patients. *L.H. Hoefsloot¹, H.G. IJntema¹, J. Schoots¹, H. Scheffer¹, N.V.A.M. Knoers².* 1) DNA Diagnostics, Dept Human Genetics, University Medical Centre, Nijmegen, Netherlands; 2) Clinical Genetics, Dept Human Genetics, University Medical Centre, Nijmegen, Netherlands.

Gitelman Syndrome is an autosomal recessive renal transport disorder, characterized by hypomagnesemia, hypokalemia, metabolic alkalosis, and hypocalciuria. Most patients suffer from carpopedal spasms, paresthesias, and fatigue. Although the disease is considered to be relatively mild, the quality of life of most patients is considerably lower than in control persons. The causative gene is the *SLC12A3* gene, which encodes the thiazide-sensitive sodium-chloride co-transporter (NCC) in the distal tubule of the renal nephron. In the past few years we have analyzed DNA samples of 93 Gitelman syndrome patients by direct sequencing of all 26 exons of the *SLC12A3* gene. In 50 (54%) patients two mutations were found. In another 16 patients one mutation was detected. This means that in 66 patients (71%) the diagnosis Gitelman syndrome was supported, and that 116/132 (88%) of the alleles have been identified. In total 43 novel mutations were identified. Of these, the majority (30) were missense mutations, the other mutations were splice site mutations (9), nonsense mutations (2), one insertion and one deletion. Furthermore, we were able to prove that a few amino acid changes which have been described before as possible mutations, are in fact polymorphisms. We conclude from these analysis that scanning the whole coding sequence of the *SLC12A3* gene is the best option to identify mutations in Gitelman Syndrome, as opposed to assaying only the known mutations.

Mutation Analysis of COL8A2 Gene in Patients with Posterior Polymorphous Corneal Dystrophy. S.M.

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Purpose: Posterior Polymorphous Corneal Dystrophy (PPCD) (MIM#122000) is an autosomal dominantly inherited endothelial dystrophy. PPCD has significant phenotypic variability and genetic heterogeneity. Mutations in *VSX1* and *COL8A2* genes have been associated with PPCD. We screened a cohort of PPCD patients for mutations in the *COL8A2* gene.

Methods: A cohort of 30 PPCD patients with diverse ethnic origin were screened for mutations in *COL8A2* by single strand conformational polymorphism analysis (SSCP). As a preliminary screen, only amplicons with previously documented mutations were analyzed. These patients were already screened for mutations in the *VSX1* gene by direct sequencing and did not have any disease causing mutation documented. Any change detected was confirmed by direct sequencing and was assessed in controls by restriction enzyme digestion.

Results: A novel missense mutation (L450W) was found in the sample of a patient with PPCD. The change occurs in a conserved amino acid residue in the important tri-helical region of the protein. The change was not present in any of 154 control chromosomes screened by restriction enzyme digestion (Bsr1).

Conclusion: We found a novel mutation in the *COL8A2* gene in our patients which confirms the former observation of *COL8A2*'s role in PPCD. The fact that no change was found in *VSX1* or *COL8A2* genes in other patients could further expand the genetic heterogeneity of PPCD.

Types and frequencies of mutations in *ABCC6* that characterize the molecular genetics of Pseudoxanthoma Elasticum. *S. Ibbá*^{1,8}, *A. Lumsden*², *U.P. Guenther*¹, *D. Foernzler*⁷, *S. Christen-Züch*³, *R. Ramesar*⁴, *M. Lebwohl*⁵, *D. Hohl*³, *K.H. Neldner*⁶, *K. Lindpaintner*⁷, *R.I. Richards*², *B. Struk*^{1,8}. 1) Max-Delbrueck Centrum, Berlin, Germany; 2) Department of Molecular Bioscience, The University of Adelaide, Australia; 3) Department of Dermatology, University of Lausanne, Switzerland; 4) Department of Human Genetics, Medical School, University of Cape Town, South Africa; 5) Department of Dermatology, Mount Sinai School of Medicine, New York, NY; 6) Department of Dermatology, Texas Tech University Health Sciences Center, Lubbock, TX; 7) F. Hoffmann-La Roche Ltd, Roche Genetics, Pharmaceuticals Division, Basel, Switzerland; 8) Charité, Franz-Volhard Clinic, HELIOS-Klinikum, Humboldt-University Berlin, Germany.

Mutations in the *ABCC6* gene have recently been found to cause pseudoxanthoma elasticum (PXE). We performed a mutation screen in *ABCC6* through direct sequencing of 170 PXE chromosomes from 81 families. We identified a total of 59 distinct mutations, of which 43 are novel variants. Considering 68 known mutations so far, this increases the total number of PXE mutations identified to 111. From 59 mutations 32 are missense, 8 are nonsense, 6 are likely splice site point mutations, 1 is a small insertion, 7 are small deletions, 5 are large deletions. Potentially disease causing mutations were uncovered in 97% (165/170) of chromosomes. This is the highest mutation detection rate for this gene. While most mutations are single variants, the mutations *R1141X*, *R1164X*, *Q378X*, the splice donor site mutation c.2787+1G>T and the large deletion comprising exons 23-29 are the most frequent PXE mutations at 26%, 5%, 3,5% 3% and 11% respectively. Phase of the PXE haplotype was established from segregation analysis of two proximal and two distal *ABCC6* flanking polymorphic markers. Most chromosomes carrying *R1141X*, *R1339C*, the IVS21 splice donor site mutation and e23-29 deletion have related haplotypes suggesting their origin from single founder mutations. The types of mutations found support loss-of-function as the molecular mechanism for the PXE phenotype, compatible with a recessive mode of inheritance.

Detection of four novel mutations in Hermansky-Pudlak syndrome type1 gene(*HPS1*). *S. Ito, T. Suzuki, K. Inagaki, N. Suzuki, Y. Tomita.* Dermatology, Nagoya University, Nagoya, Aichi, Japan.

Hermansky-Pudlak syndrome(HPS) is an autosomal recessive disorder characterized by oculocutaneous albinism(OCA), bleeding tendency due to a lack of platelet-dense granules, and lysosomal storage of ceroid-like material. The albinism results in various degrees of pigment dilution, congenital nystagmus, amblyopia, and marked iris transillumination. Life-threatening manifestations are frequent, and death typically results from restrictive lung disease in patients aged 30-50 years. So far seven subtypes of HPS, which are associated with seven genes(*HPS1*, *ADTB3A*, *HPS3*, *HPS4*, *HPS5*, *HPS6*, and *DTNBPI*), have been described. The most common type is HPS-1, and more than 18 pathological mutations in the *HPS1* gene have been reported. We screened the *HPS1* gene of 29 unclassified OCA patients with the PCR-SSCP/heteroduplex method. PCR products showing aberrant patterns were reamplified and sequenced directly. We detected nine different mutations including four novel mutations (W583X, L668P, 532insC, and del1691A), and identified eleven individuals with HPS-1. Two of the four novel mutations were frameshift mutations and one of them was nonsense mutation. These three mutations were definitely pathogenic. In case of amino acid substitution, L668P, we examined the frequency in normal Japanese. We screened 112 normally pigmented Japanese for the L668P mutant allele, and failed to detect this mutation. This indicates that the L668P missense mutation might be very rare in general Japanese population and could be defined statistically as a pathological mutation. Clinically, some HPS-1 patients had not noticed their own bleeding tendency because of the mild symptoms, and were hardly distinguished from other types of OCA by clinical feature, especially in childhood. Therefore the diagnosis of HPS-1, which enable to prognose implications and early treatment, should be made by genetic analyses.

Genetic characterization of familial hypercholesterolemia in four large Swedish pedigrees. *H. Jiao*², *S. Lind*^{1,3}, *C.M. Lindgren*², *M. Eriksson*³, *V. Mkel*², *I. Fransson*², *G. Eggertsen*¹, *J. Kere*², *B. Angelin*³. 1) Division of Clinical Chemistry, Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden; 2) Department of Bioscience at Novum, Karolinska Institutet, Stockholm, Sweden; 3) Center for Metabolism and Endocrinology, Department of Medicine and Center for Nutrition and Toxicology, Karolinska Institutet, Stockholm, Sweden.

The most common form of autosomal dominant familial hypercholesterolemia (FH) is caused by mutations in the low density lipoprotein receptor gene (LDLR). Other similar forms of this disorder are caused by mutations in the genes for apolipoprotein B-100 or the PCSK9 gene. In order to understand the genetic basis of the disease in Swedish FH families, twenty microsatellite markers representing 5 known FH-related genes, LDLR, apoB100, CYP7A1, PCSK9 and ARH, were selected for an exclusion study in 4 FH pedigrees in which no mutations in the LDLR and apoB100 genes were detected in probands by SSCP and sequence analysis. Out of the 5 genes, four were excluded by linkage analysis, but the LDLR gene showed linkage in three pedigrees with a maximum NPL score 3.6. Six patients from the 4 pedigrees were chosen for sequence analysis of the LDLR gene (the promoter region and all 18 exons). Sequence results revealed 4 pedigree-specific sequence variants in the 3 pedigrees showing linkage to the gene. One novel missense mutation Q639R showed segregation with the disease in one of the families. The mutation was not detected in 51 healthy controls. The missense mutation (D200G), previously reported, was detected in another pedigree. In the third pedigree, two affected individuals were found to carry two intronic variants (IVS6+37G>A; IVS1+56C>T). In the fourth family there was no evidence for linkage to the investigated loci. Thus, mutations in yet unidentified gene(s) may be a cause for the FH phenotype.

Mutations of NOTCH3 in Korean patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Y. Kim*^{1, 2, 3}, *K. Min*², *J.Y. Park*², *G. Kim*², *J.H. Choi*^{2, 3}, *H.W. Yoo*^{2, 3}. 1) Asan Inst for Life Sciences, Univ Ulsan College of Medicine, Seoul, Korea; 2) Genome Research Center for Birth Defects and Genetic Diseases, Asan Medical Center, Seoul, Korea; 3) Department of Pediatrics, Asan Medical Center, Seoul, Korea.

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a small vessel disease of the brain, which is characterized by recurrent subcortical ischemic attacks, stepwise or progressive cognitive decline, and white-matter abnormalities on brain magnetic resonance images. Mutations in the human Notch homologue 3 gene (*NOTCH3*) were identified as the underlying cause of CADASIL in different ethnics. The human *NOTCH3* protein contains EGF-like repeats, three Notch/Lin-12 domains, and three cdc10 ankyrin-like repeats. The EGF-like repeats reside in the extracellular domain of *NOTCH3*, and upon binding of a ligand to the EGF-like repeats, the intracellular domain of *NOTCH3* is released from the membrane to the nucleus by proteolytic cleavage and functions as a transcription regulator. So far, 56 missense mutations of *NOTCH3* have been identified, and all of them are either loss or gain of a cysteine residue in the EGF-like repeats, possibly resulting in an unpaired cysteine residue within a given EGF-like repeat in the extracellular domain. Using PCR-directed DNA sequencing analysis, we identified five heterozygous missense mutations of *NOTCH3* in 10 of 36 Korean CADASIL patients, including a novel mutation, R587C, and four reported mutations, R110C, R133C, C174R and R544C. To further confirm the association of R587C with CADASIL, we tested 140 alleles of non-patients for the presence of R587C, verifying that R587C is not present in the healthy controls. All the identified mutations were located within the EGF-like repeats. The incidence of *NOTCH3* mutations was 27.8 % in the Korean CADASIL patients, approximately similar to the incidence reported in other Asian groups. Our findings further confirm that *NOTCH3* is the defective gene in CADASIL throughout different ethnic backgrounds and that defective disulfide bridging of *NOTCH3* may be a primary cause in the pathogenesis of CADASIL.

Screening of point mutations causing α -thalassaemia using denaturing high-performance liquid chromatography (dHPLC). *H.Y. Law¹, M. Chee², E. Tan², G.P. Tan², I. Ng^{1,2}*. 1) Dept Pediatrics, KK Women's & Children's Hosp, Singapore, Singapore; 2) National Thalassaemia Registry, Ministry of Health, Singapore.

α -thalassaemia is a common genetic disorder in South East Asia. The most severe form of α -thalassaemia, Barts hydrops foetalis is not only fatal but causes complication during pregnancy. Barts are usually caused by homozygous 2-gene deletions which account for >70% of α -thalassaemia alleles in Singapore. Mutations in Cd30(GAG) and Cd59(GGC/GAC), both found in local population, have been reported to cause HbH Barts. Our mutation screening strategy thus includes a reverse dot blot (RDB) method for detection of these and 3 other point mutations (Hb Constant Spring, Hb Quong Sze, and Hb Pakse). As heterozygous carriers of these 5 mutations have borderline or normal MCV values, RDB analysis often yields negative results. To improve the efficiency of screening, we have developed a dHPLC protocol to pre-screen samples before confirmation with RDB. DHPLC screening includes specific amplification of the α globin gene followed by 3 nested PCR reactions. The 3 amplicons cover the CAAT box to middle of IVSI, Cd53 to Cd120 in exon 3, and 3 of IVSII to 30bp 3 to poly A. DHPLC analysis carried out at 65.7C, 65.4C and 64.5C for the respective amplicons detected heteroduplexes of Cd30(-GAG), Cd59(GGC/GAC), HbQS, HbCS, and HbPk. These conditions were thus used to 1) screen for 70 samples for point mutations and 2) test for sensitivity in identifying 6 other mutations in the same regions. DHPLC screening of 70 samples found 2 suspected to be carriers of HbQS and 3 of Cd59 and were confirmed by RDB. Two unusual heteroduplexes were observed. They were found to be a SNP in 3' UTR region (nt50 G/C) and IVSII sequence of 1 gene respectively by sequencing. Analysis of 6 other mutations (StCd(-T); IVSI donor site(-5nt), poly A(AATAAA/AATGAA), and 3 variant chains, HbG Waimanalo, Hb Manitoba and HbJ Singapore) found heteroduplexes readily detected in all. In conclusion, a dHPLC protocol is established to efficiently screen for mutations causing α -thalassaemia in our population. Pre-screening using dHPLC reduces the number of samples for confirmatory RDB or sequencing analyses.

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Two de novo mutations of TCOF1 gene in Taiwanese of Treacher Collins syndrome. *J. Lin.* Genetic Div, Pediatric Dept, Chang Gung Children's Hosp, Taipei, Taiwan.

Treacher Collins syndrome is a disorder of craniofacial development. The features include antimongoloid slant of the eyes, coloboma of the lid, micrognathia, microtia and other deformity of the ears, hypoplastic zygomatic arches, and macrostomia. Conductive hearing loss and cleft palate are often present (Dixon, 1996). We design 27 pairs of primers for PCR and analyzed the 25 exons of TCOF1 gene by direct sequencing in fifteen patients of craniofacial disorder. Two de novo mutations were found in two typical patients of Treacher Collins syndrome. Nt1483 del (AG) was found in exon 10 in one patient. Nt2606 del(C) located in exon 16 was detected in other patient. No mutations were found in several atypical patients. But high rate of SNP was confirmed again in TCOF1 gene and discussed.

VHL gene mutations in young individuals with polycythemia and high serum erythropoietin. *M. Martella¹, M.L. Randi², A. Casarin¹, M.C. Putti¹, G. Opocher², A. Murgia¹.* 1) Pediatrics, Univ Padua, Padua, Italy; 2) Medical And Surgical Sciences, University of Padua, Italy.

The Chuvash polycythemia is a familial congenital polycythemia with increased erythropoietin (Epo) level, which has been found to be associated with homozygosity or compound heterozygosity for mutations of the von Hippel-Lindau (VHL) gene, with the p.Arg200Thrp variant being the most frequent alteration. We have studied the VHL gene in seven young individuals, 5 unrelated and 2 siblings, with congenital sporadic polycythemia and inappropriately high Epo levels, all from north-eastern Italy. In only one patient a heterozygous VHL gene variant, never previously reported in polycythemic or in von Hippel-Lindau subjects, was identified (c. 430G>A; p.Gly144Arg). No other VHL point mutations or deletions have been detected. The 48 year-old mother of this subject, carrier of the same VHL variant, does not have erythrocytosis, and no signs of VHL disease have been detected in either individual after a thorough clinical and radiological investigation. No other siblings of the proband available for the study have been found to carry the VHL variant; the family history is negative for VHL-related manifestations or polycythemia. The causative role of this new VHL missense mutation in determining the erythrocytosis of this patient is questionable. As for other heterozygote cases reported in literature, it is possible to hypothesize that some other unknown factors, maybe a second unidentified mutation in another oxygen-sensing pathway gene, might contribute to determining the polycythemia phenotype, according to a digenic mode of inheritance. This report underscores the fact that VHL mutations may not be the only defect responsible for polycythemia due to disturbed hypoxia-sensing mechanisms.

Mechanisms of *COL5A1* Null Alleles in Classical Ehlers-Danlos Syndrome. *A.L. Mitchell, U. Schwarze, J.F. Jennings, P.H. Byers.* Univ of WA, Seattle, WA.

Classical EDS is an autosomal dominant connective tissue disorder, characterized by soft, doughy skin, hyperextensible joints, and atrophic, widened scars. The majority of cases result from abnormalities in type V collagen, a heterotrimer of two 1(V) and one 2(V) chains, which are encoded by the *COL5A1* and *COL5A2* genes, respectively. About 25% of patients are haploinsufficient for *COL5A1* expression at the RNA level (termed null alleles), as a result of mutations that lead to premature stop codons (PTCs) and subject the message to nonsense-mediated decay (NMD). We identified the genomic mutations in 9 patients with null alleles. Five affected splicing (4 were intronic and 1 was exonic), 2 were nonsense codons, and 2 were insertion/deletions. We then studied nuclear RNA from these patients to assess the effect(s) of the mutations on RNA expression. For the nonsense and frameshift mutations, as well as for 2 of the splice site mutations, there was only 1 outcome- a product with an altered reading frame that was degraded in the NMD pathway. We term these "total nulls" since all of the message from the disease allele is unstable and degraded. The other 3 splice site mutations resulted in 2 RNA products. For 2 cases, one of the products had an altered reading frame and was subject to decay, while the second maintained an intact reading frame through exon skipping and was stable. We call these "partial nulls" since only some of the product from the abnormal allele is degraded. We identified +1 donor site mutations (g>a) in 2 different introns- 19 and 35- and the outcomes differed. The IVS19 alteration resulted in use of a cryptic intronic splice site, partial intron inclusion (4 bp), and a frameshift. The IVS35 change resulted in 2 outcomes- an out-of-frame partial intron inclusion, which was degraded, and an in-frame exon skip, which was stable. From this we conclude that splice site mutations can have solely qualitative effects on mRNA (total nulls) or both qualitative and quantitative effects (partial nulls), that the outcome of a splice site mutation cannot be predicted by the genomic alteration alone, and that the phenotype may depend primarily on loss of type V collagen rather than the presence of abnormal molecules.

CFTR gene mutations and Haplotypes in Cystic Fibrosis and Chronic Rhinosinusitis Chilean patients. G.

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Cystic Fibrosis (CF) is the most prevalent lethal autosomal recessive disease in the Caucasian population (1/2500 newborns). Its prevalence in Chile is calculated to range 1/4000 to 1/2000 newborns in different regions. CF is caused by mutations in the cystic fibrosis transmembrane regulator (CFTR) gene, which encodes a protein that functions as a chloride channel. This protein regulates electrolyte and water transport through secretory cells. Failure of this protein increases viscosity of mucus and other secretions. Chronic Rhinosinusitis (CR) is a CFTR related disease, since greater viscosity of mucus is one of its causes. More than 1000 CFTR gene mutations have been described. Mutation frequency varies among different ethnic groups and clinical presentations. Twenty CFTR mutations were evaluated in 28 CF patients. Fifteen mutations were evaluated in 10 CR patients. Haplotypes using three intragenic genetic markers (M470V, T854T and IVS8T) were determined in both groups of patients. Four mutations in CF patients were found. Delta F508 was found in 18 chromosomes, G542X in 3 chromosomes, R334W in 2 chromosomes and R117H in one chromosome. Using SSCA and sequencing, we found two variants (T351S, G528G) in three chromosomes. The latest last one modifies the last base of exon 10. The most usual haplotype present in chromosomes bearing mutations was M470,T854(-),T7. In CR patients we did not find any mutation, including T5 variant in IVS8T. The percentage of detection in CF patients was 44%, less than it has been reported for the same panel of mutations in other Caucasian or Hispano-Americans populations. The absence of mutations in CR patients is also different from what has been reported in Caucasian population. Both observations may be due to Chilean population ethnic admixture. DIPUV 28/2001.

Absence of homozygosity of the common *SBDS* truncation mutation in Shwachman-Diamond syndrome. *J.A. Morrison¹, N. Richards¹, G.R.B. Boocock^{1,2}, X. Bai¹, Y. Liu¹, L. Ellis^{3,4}, P.R. Durie^{3,4,5}, J.M. Rommens^{1,2}.* 1) Program in Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Department of Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, Canada; 3) Program in Integrative Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 4) Division of Gastroenterology and Nutrition, The Hospital for Sick Children, Toronto, Ontario, Canada; 5) Department of Paediatrics, University of Toronto, Toronto, Ontario, Canada.

Shwachman-Diamond syndrome (SDS; OMIM260400) is an autosomal recessive disorder, characterized by exocrine pancreatic insufficiency, hematological dysfunction, and skeletal abnormalities. Mutations in the *SBDS* gene have been determined to be causative of SDS. *SBDS* is located at 7q11, and is comprised of 5 exons spanning 7.9 kb of genomic sequence. An unprocessed pseudogene (*SBDSP*) shares >97% identity, and occurs in a paralogous duplicon located 5.8 Mb distally. Gene conversion, leading to the incorporation of pseudogene sequences into *SBDS*, has been found to be the primary cause of SDS-related mutations. Mutation screening by restriction analysis or direct sequencing of PCR-amplified exons has been performed on patients from 171 families meeting inclusion criteria (occurrence of neutropenia and exocrine pancreatic insufficiency). Three common gene conversions have been identified in SDS patients, including a nonsense mutation (183_184TA>CT), a splice site mutation (258+2T>C) and an extended conversion encompassing both sites. Mutations have been identified in 149 families, with 90% having at least one converted allele, and 67% having two converted alleles. Twenty-six less common mutations have been found, including two exon 3 gene conversions. Despite accounting for two-thirds of disease-causing alleles, no SDS patients were homozygous for 183_184TA>CT. This mutation is predicted to cause early protein truncation; the absence of homozygosity in SDS patients indicates that *SBDS* is an essential gene, and that complete loss of function is incompatible with life.

Molecular Characterization of BBS2 in an Ethnically Diverse Canadian Population with Bardet-Biedl Syndrome.

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Purpose: Bardet-Biedl syndrome (BBS) is an autosomal recessive multi-systemic disorder. BBS ultimately leads to blindness as a result of severe retinitis pigmentosa. BBS is genetically heterogeneous with 8 loci documented. Mutations in *BBS2* are proposed to account for a minority of cases (8-16%) (Katsanis et al., 2001). We investigated the role of *BBS2* (OMIM; 606151) in an ethnically diverse Canadian population with Bardet-Biedl syndrome.

Methods: Patients diagnosed with BBS were recruited through the Hospital for Sick Children and from the Northwest Territories. Patients were screened for sequence changes in all 17 coding exons of *BBS2* using a combination of single strand conformational polymorphism (SSCP) analysis and direct cycle sequencing. Sequence changes were confirmed with restriction enzyme digestion.

Results: We have identified a combination of novel and previously described *BBS2* sequence changes in our patient cohort. Sequence changes identified include a homozygous L125R in a First Nations family, heterozygous D104A and Y24X previously described sequence changes and a novel heterozygous R703X.

Conclusion: We report novel changes in *BBS2* in patients with a BBS phenotype. The role of *BBS2* in our diverse patient cohort appears to be minor, consistent with previous reports.

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Mutation Analysis of the Spastin Gene (SPG4) and the Atlastin Gene (SPG3A) in Korean Patients with Hereditary Spastic Paraplegia. *S.Y. Park¹, C.S. Ki¹, H.J. Kim¹, D.H. Sung², B.J. Kim³, W.Y. Lee³, S.G. Lee¹, E.H. Cho¹, J.W. Kim¹.* 1) Department of Laboratory Medicine, Sungkyunkwan University School of Medicine, Samsung Medical Center, Seoul, Korea; 2) Department of Physical Medicine and Rehabilitation, Sungkyunkwan University School of Medicine, Samsung Medical Center, Seoul, Korea; 3) Department of Neurology, Sungkyunkwan University School of Medicine, Samsung Medical Center, Seoul, Korea.

Hereditary spastic paraplegia (HSP), a genetically and clinically heterogeneous group of neurodegenerative disorders, is characterized by progressive lower limb weakness and spasticity. Among the 8 loci associated with the autosomal dominant uncomplicated HSP (AD-HSP), mutations in the SPG4 gene on the chromosome band 2p22 are most common, accounting for about 40% of all cases and the SPG3A locus on chromosome 14q11-21 comprises approximately 10% of patients. In this study, 18 Korean patients clinically diagnosed with uncomplicated HSP (11 AD and 7 sporadic) were screened for mutations in the SPG4 gene by full sequencing of all coding exons and the flanking intronic sequences. Those negative for SPG4 mutation were analyzed for mutations in the SPG3A gene. We identified 8 SPG4 mutations, seven of which have not been reported elsewhere. Among the detected mutations were 3 missense mutations (I344K, R499H, and D584V), 2 in-frame deletions (1210_1212delTTT and 1137_1142delACTCTT), 2 frameshift mutations (1650_1651delAG and 1276_1277delCT), and one splice mutation (1413+3delAAGT). Additional testing with the SPG3A gene in the SPG4-negative patients did not show any mutations. This is the first comprehensive report on SPG4 mutations in Korean patients with uncomplicated AD-HSP, showing a higher mutation detection rate (7/11; 64%) than those in previous studies. On the other hand, there was only one patient with sporadic HSP carrying the SPG4 mutation (1/7; 14%), showing a good concordance to previous reports.

Truncation of HERG proteins cause Long QT Syndrome in two Dutch families. A.D.C. Paulussen¹, A. Raes², R.J. Jongbloed¹, R.A.H.J. Gilissen³, A.A. Wilde⁴, D.J. Snyders², H.J. Smeets¹, J. Aerssens³. 1) Dept Genetics & Cell Biology, Univ Maastricht, Maastricht, The Netherlands; 2) Laboratory of Molecular Biophysics, Physiology and Pharmacology, University of Antwerp, Antwerp, Belgium; 3) Dept Drug Discovery, Jonhson & Johnson Research and Development, Beerse, Belgium; 4) Experimental and Molecular Cardiology Group, Academic Medical Centre, Amsterdam, The Netherlands.

Background Long QT Syndrome (LQTS) is a ventricular arrhythmia, recognized by a prolongation on the ECG and which causes symptoms such as synopes and sudden death. Six genes have been identified for this syndrome. Mutations in one of these genes, the *KCNH2* (HERG) ion channel gene, are responsible for LQTS by causing a reduction of the delayed rectifier current I_{Kr}. This reduction delays repolarisation of cardiac cells and renders patients vulnerable to ventricular arrhythmias. *Methods and Results* We identified and characterized two heterozygous mutations (E698X and P872fs877) in the C-terminus of the *KCNH2* gene in two Dutch LQTS families. Both mutations lead to premature truncations of the C-terminus of the HERG protein. Biochemical, confocal microscopy and electrophysiological techniques were used to investigate protein expression, trafficking and function. The E698X protein, lacking 461 amino acids, was expressed with reduced quantities en produced no currents *in vitro*. The P872fs877 protein, lacking 282 amino acids was expressed and functional. P872fs877 mutant channels produced currents with biophysical properties similar to WT channels. Heterologous co-expression of WT and P872fs877 subunits showed a complex functionality; during action potential clamp experiments the capacity of hERG current was increased from ~30 percent (homologous WT or mutant expression) to 70 percent. However, this positive effect was completely abolished by a high increase in ER-retention of heterotetramers. *Conclusions* LQTS in the first family is caused by haplo-insufficiency due to absence of E698X HERG proteins. LQTS in the second family is caused by ER retention due to heterotetramerization, which has a greater impact than the apparent gain in function of mutated channels.

A novel point mutation in the KCNQ1 gene changes the channel gating kinetics and causing Long QT Syndrome.

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Long QT syndrome (LQTS) is a hereditary cardiac disorder characterized by syncope, seizures, and sudden death from ventricular arrhythmias, specifically torsade de pointes. Several ion channel genes (KCNQ1, KCNH2, SCN5A, KCNE1, and, KCNE2) are associated with LQTS, and multiple mutations have been identified. The KCNQ1 gene on chromosome 11 encodes the alpha-subunit of slowly repolarizing cardiac potassium channel, and defects in this protein cause the most common form of LQTS referred to as LQT1. Over 80 mutations have been identified in KCNQ1 to be associated with autosomal dominant LQTS. The effects of some of these mutations can be studied by cellular expression assay since the characteristics of the expressed gene define a cellular phenotype. Loss of function or defective subcellular trafficking have been revealed as the molecular pathologic mechanisms. In this study, one family with clinical evidence of LQTS was analyzed for mutations by use of dHPLC and DNA sequencing analyses for mutation screening in the KCNQ1 gene coding region. The affected members were found to have a previously unidentified, point mutation K393N in the C-terminal. This mutation changes the channel gating kinetics. Moreover, it shows a smaller instead of larger current magnitude when co-expressed with wild-type beta-subunit minK. These data suggest that this mutant may act through a different molecular pathogenic mechanism.

Detection of large deletions in the *MECP2* gene by Real-Time Quantitative PCR. *G. Raca, A. Alkhateeb, S. Das.*
Human Genetics, University of Chicago, Chicago, IL.

The molecular basis of Rett syndrome has been well established and numerous mutations in the *MECP2* gene have been reported. Approximately a quarter of patients with classic Rett syndrome however, do not have a detectable mutation within the coding region of the *MECP2* gene. Recently, large deletions in the *MECP2* gene have been identified in a proportion of patients in whom current PCR-mediated assays failed to identify molecular abnormalities. We investigated the presence of large deletions in the *MECP2* gene in a group of 180 patients with suspected Rett syndrome whose samples were sent to our DNA diagnostic laboratory for testing, and in whom no mutations were identified in the *MECP2* gene by full gene sequencing. We used a real-time quantitative PCR based assay to test for heterozygous deletions. The assay consists of simultaneous amplification of a fragment from the test gene (*MECP2*) together with a fragment from a reference gene (albumin), and the *MECP2* copy-number derived by comparing calculated concentrations of the test and the control gene. All patients were tested for two regions within the *MECP2* gene, exons 3 and 4, which lie in the region of the gene, found to be deleted, in previous reports. Deletions were excluded in 165 patients. So far deletions of exons 3 and 4 have been identified in at least 3 patients (1.6%). One patient shows results that strongly suggest the presence of a duplication within the gene. The deletion/duplication status of the remaining 11 patients is currently being investigated. We are also in the process of confirming our results by using alternative methods. Our results support the finding that deletions, and perhaps duplications, in the *MECP2* gene play an important role in patients with Rett syndrome. Deletion analysis of the *MECP2* gene should be considered in addition to mutation analysis for clinical testing purposes in patients with suspected Rett syndrome.

A Novel point mutation in NKX2.5 gene in a moroccan family with Atrioventricular conduction disturbance (AV block) and secundum atrial septal defect (ASD). *L. Rifai*¹, *A. Laamri*¹, *A. Maazouzi*², *A. Sefiani*¹. 1) Genetique medicale, Insitut National d Hygiene, Rabat, Morocco; 2) Service de Chirurgie CardioVasculaire, Hopital Ibn Sina, Maroc.

Atrial septal defect (ASD), which is found in 6 to 10 percent of all people born with congenital heart disease, usually occurs as an isolated malformation but it shows an autosomal dominant pedigree pattern in familial cases. Several mutations in NKX2.5 gene have been described and co-segregate with varied cardiac anomalies, including ASD and AtrioVentricular conduction defects, what suggests that this transcription factor contributes to various cardiac developmental pathways, and particularly to regulation of septation and maturation and maintenance of atrioventricular node function. Here we report the identification of a novel missense heterozygous mutation: a A to C transversion, resulting in a Glutamine to Proline substitution at codon 160 (Q160P) occurring in a moroccan family. This mutation co-segregates with all affected individuals screened by sequence analysis, but not with unaffected members. This mutation creates a new restriction site for Mlu113I, and was not found in 100 controle DNA samples from unrelated individuals. This mutation occurs in the Homeodomain which is a highly conserved region, and therefore its predicted to alter the DNA binding affinity, and to reduce transcription activity. This data reinforces the hypothesis according to which the NKX2.5 haplo-insufficiency causes ASD and atrioventricular node dysfunction.

Screening of the XLMR genes *ARX*, *MECP2* and *FMR2* in 200 mentally retarded males from Italy. *M. Rosati*^{1,2}, *M.G. Pomponi*¹, *G.M.E. Marino*¹, *A. Terracciano*¹, *A. Pampanos*¹, *P. Chiurazzi*¹, *G. Neri*¹. 1) Istituto di Genetica Medica, Universit Cattolica, Rome, Rome, Italy; 2) Ospedale San Giacomo, ASL RM/A, Rome, Italy.

X-Linked Mental Retardation (XLMR) is a genetically heterogeneous condition which can present in either syndromic (MRXS) a nonspecific (MRX) form. The *ARX* and the *MECP2* genes were found mutated in both MRXS and MRX cases, while expansions in the *FMR2* gene are usually associated with MRX. We screened 200 male patients with mild to severe MR for mutations in the *ARX*, *MECP2* and *FMR2* genes. All patients were negative for the amplification in the *FMR1* gene, thus excluding fragile X syndrome, and none of them had dysmorphic features. Only few (7%) had a history of seizures or infantile spasms. *ARX* mutations were screened by SSCP, using 8 primer pairs designed by us (available upon request). We found 4 cases with a silent point mutation in exon 4 (G1347T), one with a G>T transversion in intron 3 (position +81) and one *de novo* case with the 24-bp duplication, previously described by Strømme et al. [Nature Genet. (2002) 30:441-445]. This latter patient was a 4-year old child with dystonic movements of the hands, typical of Partington syndrome. The *MECP2* gene was analyzed by direct sequencing of the entire coding sequence. We found 5 silent point mutations in exon 3, one G>C transversion in intron 3 (+30) and a C1024T transition causing the previously unreported aminoacidic change P342S, which may account for the MR phenotype. Finally, no expansion was detected in the CGG repeat of the *FMR2* gene. Our results suggest a minor role for the *ARX*, *MECP2* and *FMR2* genes in causing nonspecific XLMR and suggest that other yet unidentified genes wait to be characterized.

Novel ENPP1 mutations in patients with generalized arterial calcification of infancy. *N. Ruff¹, B. Uhlenberg^{1, 2}, R. Terkeltaub³, P. Nürnberg^{1, 4}, F. Rutsch⁵.* 1) Gene Mapping Center, Max-Delbrueck-Centrum, Berlin, Germany; 2) Department of Neuropediatrics, Charite, University Medical School Berlin, Germany; 3) Department of Medicine, VAMC, UCSD, San Diego, USA; 4) Institute of Medical Genetics, Charite, University Medical School Berlin; 5) University Childrens Hospital, Muenster, Germany.

Generalized arterial calcification of infancy (GACI) is characterized by calcification of the internal elastic lamina of large and medium-sized muscular arteries and stenosis due to myointimal proliferation. Sometimes peri-articular calcification is also observed. Although survival to adulthood has been reported, most patients die within the first six months of life. Recently, we identified mutations of ENPP1 as the cause of this recessive genetic defect (OMIM 208000). ENPP1 encodes ecto-nucleotide pyrophosphatase/phosphodiesterase 1. This cell surface enzyme generates inorganic pyrophosphate, a solute that regulates cell differentiation and serves as an essential physiologic inhibitor of calcification. Mutations were shown to dramatically reduce enzyme activity. In this study, all 25 exons including their flanking splice sites and the promoter region of ENPP1 were amplified by PCR and sequenced bi-directionally from genomic DNA from patients of 12 unrelated families with GACI. We identified 11 novel homozygous or compound heterozygous mutations in 10 out of 12 unrelated patients. Thus, combined with the data of our first study, we identified a total of 24 different homozygous or compound heterozygous mutations in 18 of 23 unrelated patients. The mutations, 3 nonsense, 15 missense, 1 single amino acid deletion, and 5 frame shift mutations were scattered over the whole coding region. We identified 3 recurrent mutations, 7 x P305T, 2 x R774C, and 2 x R888W. Haplotype analysis suggests a founder effect of British extraction for mutation P305T. In contrast, mutations R774C and R888W were CpG-to-TpG mutations presumably caused by spontaneous desamination of 5-methyl cytosine. This study confirms the role of ENPP1 mutations as the main cause of GACI and reveals a broad spectrum of different mutations in GACI patients.

Novel mutations in the *POMGnT1* gene in Muscle-Eye-Brain disease. A. Saarinen¹, C. Diesen¹, H. Pihko², W.B. Dobyns³, T. Joensuu¹, A.-E. Lehesjoki¹. 1) Folkhälsan Institute of Genetics, Department of Medical Genetics and Neuroscience Center, Biomedicum Helsinki, University of Helsinki, Finland; 2) Department of Pediatric Neurology, University of Helsinki, Finland; 3) Departments of Human Genetics, Neurology and Pediatrics, University of Chicago, IL, USA.

Muscle-Eye-Brain disease (MEB) is an autosomal recessive disorder characterized by brain malformation, congenital muscular dystrophy and ocular abnormalities. It occurs worldwide but is enriched in the Finnish population due to a founder effect. Mutations in the *POMGnT1* gene encoding protein *O*-linked mannose 1,2-*N*-acetylglucosaminyltransferase 1 (POMGnT1) underlie MEB. POMGnT1 catalyzes glycosylation of proteins by transferring GlcNAc to *O*-mannose. The *POMGnT1* gene was analyzed for mutations by sequencing in a panel of 19 Finnish and 14 non-Finnish patients. We identified nine novel *POMGnT1* mutations, adding the number of reported MEB-associated mutations to 22. Three of the mutations found in this study predict protein truncation, and four altered splice products, while two are missense mutations. In addition, we found that a mutation reported previously in three patients, c.1539+1G>A that results in an inframe deletion of 42 amino acids, is the prevalent mutation in the Finnish MEB patients. Compatible with the haplotype data in Finnish MEB patients, the founder mutation accounts for 99% of the disease chromosomes in Finland. The clinical phenotypes of the non-Finnish patients in this study and published previously fall within the variation observed in the Finnish patients that are homozygous for the founder mutation. This indicates that, in addition to mutations in the *POMGnT1* gene, other genetic and environmental factors influence the MEB phenotype.

Homozygosity mapping in Turkish variant late infantile neuronal ceroid lipofuscinosis. *E. Siintola*¹, *M. Topcu*², *T. Salonen*¹, *T. Joensuu*¹, *A. Kohlschütter*³, *A.-K. Anttonen*¹, *A.-E. Lehesjoki*¹. 1) Folkhälsan Institute of Genetics, Department of Medical Genetics and Neuroscience Center, Biomedicum Helsinki, University of Helsinki, Finland; 2) Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey; 3) Children's Hospital, University of Hamburg, Germany.

The neuronal ceroid lipofuscinoses (NCLs) are a group of autosomal recessive neurodegenerative lysosomal storage diseases characterized by the accumulation of autofluorescent storage material in several cell types, especially in neurons. Clinical features include epileptic seizures, progressive psychomotor deterioration, visual failure, variable age of onset and usually premature death. At least seven subtypes of childhood-onset NCLs have been identified. The NCLs with late infantile onset (LINCL) are genetically heterogeneous with three underlying genes identified (*CLN2*, *CLN5* and *CLN6*). A variant form of LINCL present in Turkish patients has been considered a distinct clinical and genetic entity (*CLN7*). However, we recently showed that mutations in the *CLN8* gene account for a subset of Turkish variant LINCL. Our aim is to identify the *CLN7* gene in a group of ten inbred Turkish variant LINCL families by using homozygosity mapping.

As a first step, we have screened all the currently known human and animal NCL loci, *CLN1*, *CLN2*, *CLN3*, *CLN5*, *CLN6*, *CLN8*, *Cathepsin D* and *CLCN3*, for homozygosity using fluorescently labelled microsatellite markers flanking each locus in the Turkish variant LINCL family panel. Homozygosity was observed for *CLN3* and *CLN6* in patients from two families each. The *CLN3* and *CLN6* genes were sequenced in respective patients. No mutations were identified in *CLN3*. Two putative mutations were identified in *CLN6* indicating that Turkish variant LINCL may be caused by mutations in the *CLN6* gene in addition to *CLN8*. A genome-wide scan to identify the true *CLN7* locus and gene will be initiated in the remaining eight families.

Genetic analysis of the progressive familial intrahepatic cholestases. *S.S. Strautnieks¹, J.A. Byrne¹, G.M. Mieli-Vergani¹, A.S. Knisely¹, L.N. Bull², R.J. Thompson¹.* 1) Institute of Liver studies, King's College Hospital, London, UK; 2) UCSF Liver Center Laboratory, San Francisco General Hospital, San Francisco, USA.

The progressive familial intrahepatic cholestases (PFIC) are a heterogeneous group of conditions characterised by defects in the various proteins, which collectively produce bile. They are amongst the main indications for paediatric liver transplantation worldwide. The common features are presentation in childhood of a subsequently progressive cholestasis leading to fibrosis and end stage liver disease by the second decade. The incidence is 1/70,000 UK births and 2 main types of low / normal gamma GT PFIC are recognised: bile salt export pump (BSEP) deficiency and familial intrahepatic cholestasis 1 (FIC1) deficiency. Both show autosomal recessive inheritance and are caused by mutations in the *ABCB11* and *ATP8B1* genes on chromosomes 2q24-31 and 18q21 respectively. Over 500 patients have been accepted for genetic analysis at KCH / UCSF in the last 5 years. Haplotype analyses in several populations indicate that BSEP deficiency is prevalent, comprising some 40% of cases, FIC1 deficiency some 20%. A significant proportion (up to 20%) of families are unlinked suggesting further PFIC loci. Mutation analysis of *ABCB11* shows that the majority of patients are compound heterozygotes and to date 52 different mutations have been identified, on 139 mutant alleles, in 95 BSEP deficient patients. These include a panel of fifteen common or recurring mutations: the missense changes E297G, D482G, and A588V which have been found on 72 mutant alleles in 52 European patients, and twelve changes associated with hypermutable CpG sites which recur in all populations. The remaining mutations comprise: 21 missense / termination changes, 4 splice site changes, and 13 insertions and deletions which include 2 whole gene deletions. Genetic screening allows both definitive diagnosis and the provision of genetic counselling. Genotype is also a major prognostic indicator for therapeutic interventions such as biliary diversion and ileal exclusion that may delay or even prevent the need for transplantation in these conditions.

Mutation screening of *FZD4* in familial exudative vitreoretinopathy (FEVR) patients. *C. Toomes¹, H.M. Bottomley¹, S. Scott¹, D.A. Mackey², J.E. Craig³, L.M. Downey¹, C.F. Inglehearn¹.* 1) Molecular Medicine Unit, University of Leeds, Leeds, United Kingdom; 2) Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital, Melbourne, Australia; 3) Department of Ophthalmology, Flinders Medical Centre, Adelaide, Australia.

Familial exudative vitreoretinopathy (FEVR) is an autosomal dominant disorder which affects the development of the retinal vasculature. Autosomal dominant FEVR is genetically heterogeneous with at least four different genes responsible for the disorder. Two of these genes have been identified, *FZD4* which encodes Frizzled-4 and *LRP5* which encodes low-density-lipoprotein receptor related-protein 5. In this study we screened *FZD4* in a panel of 60 FEVR patients to ascertain the type, frequency and location of mutations within this gene. We used primers to amplify the two *FZD4* exons and flanking intronic sequences from genomic DNA and screened these by direct sequencing. This work is still ongoing but to date we have identified over twelve mutations within *FZD4*. These include deletions (957delG, 1498delA, 1501delCT), nonsense mutations (Q505X) and missense mutations (G36D, M105T, M157V and S497F). None of these mutations were present in 200 control individuals. These results indicate that mutations within *FZD4* are responsible for only 20% of FEVR and suggest that other autosomal dominant FEVR loci may make a larger contribution to this condition than previously thought.

Mutations of the PTPN11 gene among Finnish Noonan syndrome patients. *M. Vaisanen¹, S. Ylönen¹, A. Mannermaa^{1, 2}, M. Somer³, S. Kivirikko³, E. Kajantie⁴, J. Körkkö¹, J. Ignatius¹.* 1) Dept. of Clinical Genetics, Oulu University Hospital and University of Oulu, Oulu, Finland; 2) Dept. of Clinical Pathology and Forensic Medicine, Kuopio University Hospital and University of Kuopio, Kuopio, Finland; 3) Family Federation of Finland, Helsinki, Finland; 4) Hospital for Children and Adolescents, Helsinki University Central Hospital, Helsinki, Finland.

Noonan syndrome (NS) (MIM 163950) is a developmental disorder characterized by typical facial dysmorphism, short stature, cardiac defects and skeletal malformations. Mutations in PTPN11, the gene encoding the non-receptor-type protein tyrosine phosphatase SHP-2, have been reported to cause NS. This study aimed to screen for mutations in the PTPN11 in Finnish NS patients. We screened for sequence alterations in exons (2-15) and splice regions of the PTPN11 gene by using PCR-CSGE and direct sequencing in 20 unrelated Finnish patients with typical Noonan symptoms and some of their family members. We identified missense mutations from four patients. The mutation Asn308Asp, the most common mutation described in Noonan patients, was found from two patients. The other two mutations were Asn308Thr and Thr73Ile. This study further emphasizes the importance of codon 308 mutations in Noonan syndrome, as three out of four of the found mutations affected it. Although Asn308Thr has not been described in other studies, our study shows that a PTPN11 mutation spectrum in Finnish NS patients does not differ from those in other populations. Interestingly, mutations were found from only 20% of the NS patients compared to estimates of ~50%, which suggests an important role for mutations in other genes in Finnish NS patients.

Mutations in the newly discovered coding exon 1 of *MECP2* are a rare cause of classic Rett syndrome. I. Van den Veyver^{1,2}, R. Amir¹, P. Fang², Z. Yu¹, D. Glaze³, A. Percy⁴, H. Zoghbi^{2,3,5}, B. Roa². 1) Dept Ob/Gyn; 2) Dept Molec Hum Genet; 3) and Dept Pediatr, Baylor Coll Med, Houston, TX; 4) Dept Pediatr, Univ Alabama, Birmingham, AL; 5) HHMI, Baylor Coll Med, Houston, TX.

About 85% of individuals with Rett syndrome (RTT) have a mutation in the coding sequence of exons 3 or 4 of *MECP2* and some have large genomic deletions. However, the mutations in the remainder are still unknown. An alternative *MECP2* splice variant (*MECP2B/MECP2*) has been discovered which encodes a MeCP2 isoform that excludes exon 2 but contains a novel N-terminus encoded by exon 1. We searched for mutations in exon 1 in genomic DNA of 63 patients, 38 of whom have classic RTT and 25 have atypical RTT. Mutations of coding exons 2, 3 and 4 were previously excluded. The 38 classic RTT patients were from a total cohort of 321 previously analyzed patients. We identified a *de novo* 2-base pair (bp) deletion at the splice donor site of intron 1 (c.62+1delGT, numbered according to the ATG of *MECP2B*). This patient has classic RTT but with still some preservation of eye contact. X-chromosome inactivation (XCI) studies on leukocyte-derived DNA revealed 68%:32% skewing with the maternal X chromosome being preferentially active, possibly explaining her slightly milder phenotype. We did not detect aberrantly spliced transcripts by RT-PCR analysis on leukocyte-derived RNA, although their presence can not be excluded given the pattern of XCI. The second identified mutation in a patient with atypical Rett syndrome is an 11 bp deletion (c.47_57del) which creates a frameshift and premature stop codon (p.Gly16GlufsX36). We also discovered a 3 bp in-frame insertion (c.47_48insAGG) in a girl with classic Rett syndrome. The significance of this sequence variant is currently unclear. We conclude that exon 1 mutations are present in 0.3% (1/321) of all classic Rett syndrome cases. In samples from RTT patients (classic and variant forms combined) who were negative by prior mutation analysis, we found mutations in exon 1 in 3.1% (2/63). Although mutations in exon 1 of *MECP2* comprise a small percentage of RTT cases, analysis of this exon should be incorporated for a complete DNA diagnostic testing strategy for Rett syndrome.

Large deletions/rearrangements of the *MECP2* gene were found in clinical diagnosed Rett syndrome patients using Multiplex-Ligation-PCR-analysis (MLPA). *M. Van Ghelue*¹, *A. Steinkjer*², *T. Rath Olsen*³, *C. Jonsrud*¹, *O. Skjeldal*². 1) Department of Medical Genetics, University Hospital, UNN, Tromsø, Norway; 2) Department of Pediatric Neurology, University Hospital, Oslo, Norway; 3) Child Habilitation Centre, University Hospital, UNN, Tromsø, Norway.

Rett syndrome (MIM# 312750) is an X-linked dominant neuro-developmental disorder and a leading cause of mental retardation in females. In the large majority of females that have Rett syndrome the gene encoding methyl-CpG binding protein 2 (*MECP2*) is mutated. However, despite routine sequence based screening of the coding exons and adjacent intronic sequences, mutations are not been identified in about 10-15 % of the patients. One possible reason could be that a second gene/locus might be involved in Rett syndrome. Another possibility is that mutations may occur within regulatory regions of *MECP2*, which are not routinely screened. Yet another explanation may be the occurrence of larger deletions that may be missed by routine PCR-based screening.

In the present study we investigated clinical diagnosed Rett syndrome patients, which did not have mutations within the *MECP2* coding region as determined by a sequence based screening method. However using multiplex-ligation PCR analysis (MLPA), we were able to identify the molecular cause of several individuals within this patient group. Results of the MLPA investigations and the characterization of the deletions/rearrangements will be presented.

Two novel de novo mutations in the Na⁺, K⁺-ATPase pump gene ATP1A2 associated with Familial Hemiplegic Migraine type 2. *K.R.J. Vanmolkot¹, V. Barone², U. Turk³, E.E. Kors⁴, D. Turkdogan⁵, S. Kheradmand Kia¹, D.F. Black⁶, J. Haan^{4, 7}, R.R. Frants¹, M.D. Ferrari⁴, G. Casari², A.M.J.M. van den Maagdenberg^{1, 4}.* 1) Dpt Human Genetics, Leiden University Medical Centre, Leiden, The Netherlands; 2) Human Molecular Genetics Unit, Dibit-Dan Raffaele Scientific Institute, Milan, Italy; 3) Dpt Neurology, Dr. Ltfi Kirdar State Hospital, Maltepe, Istanbul, Turkey; 4) Dpt Neurology, Leiden University Medical Centre, Leiden, The Netherlands; 5) Dpt Pediatric Neurology, Institute of Neurological Sciences, Maltepe, Istanbul, Turkey; 6) Dpt Neurology, The Mayo Clinic, Rochester, MD, US; 7) Dpt Neurology, Rijnland Hospital, Leiderdorp, The Netherlands.

Migraine is a common neurovascular disorder characterised by attacks of severe headache and autonomic and neurological symptoms, affecting up to 12% of males and 24% of females in the general population. Familial hemiplegic migraine (FHM) is a rare autosomal dominant subtype of migraine with aura, associated with hemiparesis. The first gene identified in families with familial hemiplegic migraine (FHM1), namely CACNA1A, encodes a P/Q-type calcium channel subunit. Recently, missense mutations in the ATP1A2 gene, encoding an α -subunit of the Na⁺,K⁺-ATPase were reported for the second FHM locus (FHM2) on chromosome 1q23. Here we describe two novel de novo missense mutations, V628M and R593W, in the ATP1A2 gene in a Turkish family and a Dutch family with pure FHM. Functional analysis showed a loss-of-function effect for both mutations. Furthermore, two monozygotic twin brothers that were V628M carriers showed variation of clinical symptoms, illustrating environmental influence on the FHM2 phenotype.

Identification of deletions in patients with spinal muscular atrophy (SMA) type I, II and III and determination of gene copy numbers using MLPA analysis. *Y.J. Vos¹, H.H. Lemmink¹, A.H. v.d. Hout¹, J. Dijkhuis¹, C. Lummen¹, J.P. Schouten², C.H.C.M. Buys¹.* 1) Department of Clinical Genetics, Groningen University Hospital, Groningen, The Netherlands; 2) MRC Holland, Amsterdam, The Netherlands.

In about 95% of patients with spinal muscular atrophy (SMA) mutations have been identified in the SMN1 gene localized at chromosome 5q12-13. SMA patients are clinically distinct and classified into three different types. The clinical phenotype varies between the lethal SMA type I to the intermediate type II and the milder type III. Almost 95% of patients of all three clinical types have a homozygous deletion of at least exon 7 of the SMN1 gene. A very similar gene, SMN2, centromeric to SMN1, has been proposed as a modifying gene, since patients with the milder forms of SMA appear to have more SMN2 gene copies. Additionally, there is a clear preponderance of homozygous NAIP (neuronal apoptosis inhibitor protein) gene deletions in SMA type I patients. A novel multiplex ligation-dependent probe (MLPA) assay has been adapted by us to detect SMN1, SMN2, and NAIP gene deletions and to quantify gene copy numbers. A total of 19 SMA type I, 30 type II and 27 type III patients were tested and in all these cases at least exon 7 of SMN1 was absent. The determination of SMN1, SMN2 and NAIP gene copy numbers using MLPA has been validated by comparison with results from controls and patients obtained by dosimetric exon 7 SMN analysis and Pulsed Field Gel Electrophoresis. Upon MLPA analysis around 84% of type I, 93% of type II and 81% of SMA type III patients carried a total of 2, 3 and 4 SMN2 gene copies, respectively, and 74% of type I, 70% of type II and 55% of type III patients carried a total of 0, 1 and 2 NAIP gene copies, respectively. We conclude from our study that the numbers of SMN2 and NAIP gene copies are associated with the SMA phenotype.

Somatic and gonadal mosaicism in Hutchinson-Gilford progeria. *W. Wuyts¹, M. Biervliet¹, E. Reyniers¹, G. Novelli², K. Storm¹.* 1) Dept of Medical Genetics, University and University Hospital of Antwerp, Antwerp, Belgium; 2) Dept of Biopathology, Tor Vergata University of Rome, Rome, Italy.

Hutchinson-Gilford progeria syndrome (HGPS) is a very rare disorder characterized by acceleration of the aging process. All molecular characterized HGPS cases described so far result from de novo LMNA mutations, mostly originating on the paternal allele. In almost all patients, HGPS is caused by a 1824 C>T (G608G) mutation, leading to the activation of a cryptic splice donor site, resulting in the deletion of 150 bp at the mRNA level and the formation of a truncated LMNA protein. We describe a young boy with typical HGPS phenotype including failure to thrive, alopecia, skeletal abnormalities and sclerodermatous skin changes. Molecular analysis of the LMNA gene showed the presence of the 1824 C>T (G608G) mutation. Screening of both parents showed a wild type sequence in the father but surprisingly a weak signal corresponding to the mutant sequence was observed in the mother's leukocyte DNA, suggesting somatic mosaicism. Molecular characterization and segregation analysis of a 3' UTR G/A SNP located 566 bp after the stop codon further confirmed that the mutation in the proband resides on the maternal allele. Further studies also showed mosaicism for the 1824 C>T mutation in buccal cells of the mother with a similar ratio of mutant versus wild type of 1/10. No mutated allele could be detected in DNA from cultured fibroblasts and hair roots. Clinical examination of the mother did not reveal any clinical abnormalities. This is the first reported case of somatic and germline mosaicism for HGPS and it demonstrates the necessity for screening of both parents of HGPS patients as the presence of germline mosaicism has great implications towards the recurrence risk.

Mutational analyses in Japanese patients with *FGD-1*-linked Aarskog-Scott syndrome. *K. Yanagi*¹, *T. Kaname*¹, *Y. Chinen*², *K. Naritomi*¹. 1) Medical Genetics , University of the Ryukyus , Nishihara, Okinawa, Japan; 2) Paediatrics, University of the Ryukyus , Nishihara, Okinawa, Japan.

Faciogenital dysplasia 1 (*FGD-1*) gene has been identified as a responsible gene for Aarskog-Scott syndrome (AAS). A mutational analysis of the *FGD-1* gene was performed in six Japanese patients with AAS (four familial and two sporadic cases). We found three point mutations; i.e. two missense mutations in the exon 11 (1906C>T) and exon 15 (2221G>T), and a mutation at first position of 5 splice donor site of the intron 14 (IVS14+1, G>A), reducing *FGD-1* gene expression. As indicated previously, no genotype/phenotype correlations were further confirmed by this study. This is the first report of a mutation analysis in non-Caucasian *FGD-1* - linked AAS patients.

MUTATIONS IN THE *IRF6* GENE IN TWO PATIENTS WITH POPLITEAL PTERYGIUM SYNDROME.

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Popliteal pterygium syndrome (PPS) is a rare autosomal dominant disorder, characterized by popliteal webbing, cleft lip and/or cleft palate, lower lip pits, syndactyly, genital anomalies and pyramidal skin folds in halluces nails. Families with PPS have been linked to chromosome 1q32-q41, the critical region for the van der Woude syndrome (VWS1), suggesting that these two disorders are allelic. Mutations in *IRF6* gene have been identified in families affected with PPS and families affected with VWS. In this study, we describe two unrelated cases with clinical signs suggestive of the PPS with mutation in *IRF6* gene. Case 1: sporadic, cleft lip and palate, popliteal webs, syndactyly of toes, pyramidal wedge of soft tissue overlying the first toenails, ectopic testes, and misplaced anus. Case 2: familial, cleft lip and palate, lower lip pits, syndactyly of toes, pyramidal wedge of soft tissue overlying the second toenails. Mother of the case 2: cleft lip and palate and lower lip pits. Sequencing analysis of *IRF6* gene: mutations c250G>T and IVS3-5C>G in case 1; mutation c182C>A in case 2 and in his mother. The mutation IVS3-5C>G represents a SNP, with a frequency of 19% in our population, while the mutation c250G>T, leading to the substitution of the residue Arginine to Cysteine, at position 84, was described in other 5 patients with SPP. Except by absence of lower lip pits, the case 1 presented typical PPS. The mutation c182C>A, detected in case 2 and in his mother, leads to a predicted amino acid residue change from Alanine to Aspartic Acid at position 61. The substitution of this same amino acid was observed, previously, in one individual with VWS1. Interestingly, case 2 presents phenotypic features of both PPS and VWS1, while his mother presents typical VWS1. These data favors that the mutation in the amino acid 84 is highly correlated with the PPS phenotype while the change in the amino acid 61 might cause both diseases, even within the same family. It will be important to elucidate the molecular mechanisms leading to these clinical differences. FAPESP/CEPID.

Pathogenetic studies of the Hutchinson-Gilford progeria syndrome (HGPS). *W. Brown, W. Ju, A. Potempska, J. Shen, S. Li, P. Mehta, D. Miller, G. Merz, G. Wen, N. Zhong.* NYS Inst Basic Research, Staten Island, NY.

HGPS is a very rare premature aging syndrome with autosomal dominant inheritance. Recently, HGPS has been found to result from genetic defects of the LMNA gene, which encodes nuclear envelope proteins lamin A/C. A silent mutation, c2063CT that results in G608G, generates a cryptic splicing site in exon 11 of LMNA, and consequently truncates 50 amino acids near the C-terminus of lamin A, has been identified in ~80% of HGPS alleles. This has led us to explore the pathogenic mechanism driving the development of the HGPS as well as the molecular basis of other laminopathies. Our studies demonstrate dominant negative effects of the mutant protein progerin. It produces irregular nuclear structures, thickened abnormalities of the inner nuclear membrane, reduced nuclear pore complexes, and increased ribosome numbers. Ultrastructural analysis showed large vacuous structures within mitochondria within progeria but not control cells. Analysis of mitochondrial membrane function using an apoptotic marker of membrane potential showed significant differences between progeria cells (40%) and normal controls (73%). We have generated a progerin-specific polyclonal antibody, which detects only progerin from progeria cells and nothing from control. By comparison an anti-lamin A/C monoclonal antibody detects both progerin and the normal lamin A/C in progeria cells. Using this antibody, we are now able to localize the mutant protein within progeria cells. This approach will be useful to help understand the pathogenesis of HGPS.

Homozygous SMN1 deletions in unaffected family members and modification of the phenotype by SMN2. *T.W. Prior¹, K. Swoboda², H.D. Scott³, A.Q. Hejmanowski¹*. 1) Pathology, Ohio State University, Columbus, OH; 2) Neurology, University of Utah, Salt Lake City, UT; 3) Medicine, Brown Medical School, Providence, RI.

Spinal muscular atrophy is a common autosomal recessive neuromuscular disorder caused by the homozygous loss of the SMN1 gene. The absence of the SMN1 gene has been shown to occur in all types of SMA, childhood and adult forms. In rare cases, asymptomatic family members have also been found with homozygous mutations in the SMN1 gene, suggesting a role for phenotypic modifiers. We describe three unrelated asymptomatic individuals, with family histories of SMA, who were shown to have the homozygous SMN1 deletion. Quantitative studies indicated that the three individuals all had increased SMN2 copy numbers. In one of the cases we describe sibs with homozygous deletions with different SMN2 levels and discordant phenotypes. These cases not only support the role of SMN2 in modifying the phenotype, but our data also demonstrate that expression levels consistent with 5 copies of the SMN2 genes maybe enough to compensate for the absence of the SMN1 gene. It is also important to correctly distinguish the asymptomatic homozygous from the standard heterozygous carriers, since there is an increased recurrence risk of affected offspring and all of their offspring will be carriers. Lastly, in cases similarly to the ones described, the measurement of the SMN2 gene copy number may provide valuable prognostic information.

Studies on Cultured Myoblasts from Patients with Limb Girdle Muscular Dystrophy type 2H (LGMD2H): TRIM32 and Vesicular Aggregation. *K. Wrogemann¹, G.L. Stelmak¹, A.J. Halayko¹, E. Shoubridge², J.D. Berry¹, C.R. Greenberg¹, S. Pind¹, P. Hazelton¹, P. Frosk¹.* 1) University of Manitoba, Winnipeg, MB, Canada; 2) Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada.

Limb girdle muscular dystrophy type 2H (LGMD2H) is an autosomal recessive myopathy with a high prevalence in the North American Hutterite population. Our laboratory has mapped the gene for LGMD2H to chromosome region 9q31-33 and identified the causative mutation as D487N in the TRIM32 gene (AJHG 70:663-672). The TRIM32 protein belongs to a family of proteins believed to be ubiquitin ligases. In an effort to delineate the pathogenesis of LGMD2H we have produced semi-transformed myoblast cell lines from both normal and affected individuals. We have found that compared to the normal myoblasts there is a consistent vesicular aggregation in myoblasts from two separate LGMD2H patients. Cells probed with our anti-TRIM32 antibody by fluorescence microscopy show the association of TRIM32 with large cytoplasmic bodies, but no difference is apparent between normal and affected cell lines. The same pattern is also found in mouse skeletal muscle myoblasts, dog smooth muscle myoblasts, human skin fibroblasts, and baby hamster kidney cells. Preliminary results using immunogold electron microscopy on partially purified TRIM32 suggest that these bodies are approximately 40-50 nm in size. Immunoblot indicates that TRIM32 may be ubiquitinated, a characteristic feature of many ubiquitin ligases, often due to auto-ubiquitination. Interestingly, we have also discovered an apparent absence of these cytoplasmic bodies containing TRIM32 in myoblasts that have been induced to form myotubes. This coincides with a relatively weak immunohistochemical staining for TRIM32 in mature skeletal muscle sections. The data suggest that TRIM32 is involved in the ubiquitin proteasome system and is more important in muscle precursor cells rather than in mature muscle, indicative of a role in development / regeneration. At this time it is unclear whether the vesicular phenotype that we have identified in the LGMD2H myoblasts is directly related to this role or is a further downstream effect of the TRIM32 mutation.

Molecular basis of a temperature-sensitive PEX13 mutation of peroxisomal biogenesis disorder (2): 3D-structure and folding of the protein.

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We have revealed that a patient with NALD phenotype carried a missense mutation of PEX13 (I326T within a SH3 domain). He showed normal development just before a sudden deterioration with a high fever due to a respiratory infection and fibroblasts of the patient showed a temperature-sensitive (TS) phenotype in peroxisome assembly. As a first step to study its mechanism, we have analyzed the protein phenotype of the human (Hs) Pex13p-SH3 domain using immunohistochemical and biochemical analyses suggesting the fragility of the mutant protein (See Hashimoto et al. ASHG 2004). However, the precise molecular basis of the TS phenotype at a high temperature still remains unknown. To elucidate the mechanism, we have analyzed the 3D-structure and folding of the protein using fluorescence, circular dichroism (CD), multi-dimensional nuclear magnetic resonance (NMR), and bioinformatics. Three kinds of recombinant proteins, wild, natural mutant (I326T), and artificial mutants (I326F, I326L, I326V, I326A), were prepared. The structural analyses revealed that the recombinant HsPex13p-SH3 domain had a beta-barrel structure, which is typical for SH3 domain. Fluorescence and CD of the proteins indicate the different sub-structures of the domain. The melting temperature and folding-unfolding balances of the mutants were significantly lower than those of the wild. From these analyses, the temperature-sensitive nature of the mutation I326T in HsPex13p should be recognized, not only as simple fragility, such as susceptibility to the proteases of the native protein, but also as an imbalance of folding and unfolding kinetics, which results in an increase of unfolded proteins even at a lower temperature. Mutation effects of non-catalytic residues in genetic diseases have been implicated simply as protein degradation by structural instability. However, as shown in this study, which has directly revealed the folding state of protein in the TS-phenotype mutation, more precise investigations utilizing structural analyses could reveal the true nature of the mutations; giving a better understanding of genotype-phenotype correlation and assisting the development of new therapeutic approaches including the use of chemical chaperones.

Common TagSNPs Haplotypes of Genes Association with Fluvastatin Treatment in Familial

Hypercholesterolemic Patients. *D. Bercovich¹, S. Korem¹, Y. Friedlander², A. Houminer², A. Hoffman³, L. Kleinberg³, C. Shochat¹, E. Leitersdorf⁴, V. Meiner⁵.* 1) HMG & Pharm, MIGAL-Galille Bio-Technology Ctr, Israel; 2) Dept of Social Medicine, Heb Univ; 3) School of Pharmacy; 4) Dept of Medicine B; 5) Dept of HG, Hadassah-University Hospital, Jerusalem, Israel.

Familial hypercholesterolemia (FH) patients are commonly treated with HMG CoA reductase inhibitors. Yet, therapeutic response is frequently variable also among patients with identical LDL receptor mutations. This heterogeneity can result from differences in pharmacodynamic (PD) and pharmacokinetic (PK) parameters, which may be genetically determined. We analyzed SNPs in the CETP and the MDR1, representing PD and PK determinants respectively, and their association with variable response to fluvastatin in 77 molecularly characterized FH patients. Lipid levels were determined in a compliance-monitored clinical study at baseline and following 16 weeks of treatment with escalating doses of fluvastatin. CETP and MDR1 SNP genotyping was performed using DHPLC and sequence analysis. Linear regression was used to examine the associations between common SNPs and haplotypes (based on tagSNPs) and lipids response. Treatment with Fluvastatin resulted in mean LDL-C reduction of 21.48%; mean triglyceride (TG) reduction of 8.33%; and a mean HDL-C increase of 13.42%. Five tagSNPs in both genes were used to reconstruct 5 and 6 haplotypes accounting for 71.4% and 90.2% of the observed haplotypes in the CETP and MDR1 genes, respectively. An increase in LDL response was associated with CETP-H13 (-29.38% p=0.026) and with MDR1-h4 (-26.56% p=0.025). Similarly, CETP-H5 was shown to be significantly associated with decreased TG and HDL-C response, while MDR1-h10 was associated with a decrease in TG response. A multivariate regression model indicated an independent additive effect of CETP-H5 and MDR1-h10 on the level of TG response. SNP-haplotypes in CETP and MDR1 have significant independent effects on lipid changes following a Fluvastatin treatment in FH patients. Further studies on genetic determinants correlated with adverse-effects to Statin treatment of FH patients are underway.

A new polymorphism in chloride channel gene 2. *K.W. Kimberley¹, F.C. Britton², J.R. Hume³, H.H. Hobart¹, C.A. Morris¹.* 1) Dept. of Pediatrics/Division of Genetics, U. of Nevada School of Medicine, Las Vegas, NV; 2) Dept. of Physiology and Cell Biology, U. of Nevada School of Medicine, Reno, NV; 3) Dept. of Pharmacology, U. of Nevada School of Medicine, Reno, NV.

Cardiac arrhythmias, disturbances in the electrical conduction of the heart, are responsible for most of the 250,000 cases of sudden cardiac deaths that occur each year. Ion channels are responsible for the initiation and conduction of the electrical activity in the heart. Presently, 9 cation channel genes and 1 non-ion channel gene have been identified as causes of cardiac arrhythmias. Anion channels have also been shown to play a role in cardiac electrical conductance. Chloride channel gene 2 (CLCN2) encodes outward rectifying, volume-sensitive chloride channels when expressed in mammalian cells. To date, 25 polymorphisms have been identified in this gene: 5 in exons, 20 in introns. We screened CLCN2 to look for mutations that may be associated with cardiac arrhythmias.

We studied 100 DNA samples from individuals with LQT and other forms of arrhythmia with no mutations in the known LQT genes. Each exon of CLCN2 was amplified using PCR and analyzed by SSCP. Anomalous bands were cut out and the DNA extracted. The extracted DNA and corresponding patient sample were re-amplified and sequenced.

A new polymorphism was identified in 4 of the samples tested (2 with sick sinus syndrome, 1 with 2 syncopal episodes, QTc=0.42, and 1 normal control with 1 syncopal episode during exercise). Sequencing revealed a C to T base pair substitution at position 119 of exon 15. This mutation occurs in the third position of the codon but does not change the amino acid. However, this exon is in the splice region and the base change could affect splicing even though the amino acid is unchanged. Functional studies will be required to determine what effect, if any, this base pair substitution has on splicing and subsequent chloride channel function.

Genomic Structure and Organization of the High Grade Myopia-2 Locus (MYP2) Critical Region: Mutation Screening of 9 Positional Candidate Genes. *G.S. Scavello Jr¹, P.C. Paluru¹, J. Zhou¹, P.S. White^{2,3}, R.S. Spielman⁴, W.R. Ganter¹, T.L. Young¹.* 1) Divisions Ophthalmology and Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Division of Oncology, The Children's Hospital of Philadelphia, Philadelphia, PA; 3) Department of Pediatrics, University of Pennsylvania, Philadelphia, PA; 4) Department of Genetics, University of Pennsylvania, Philadelphia, PA.

Autosomal dominant high myopia is a common complex eye disorder, with implications for blindness due to increased risk of retinal detachment, chorioretinal degeneration, premature cataracts, and glaucoma. An interval of 2.2 cM was defined on chromosome 18p11.31 using 7 families with AD high myopia and was designated the MYP2 locus. We sought to characterize the causative gene(s) by direct sequencing of 9 known positional candidate genes in the region. The BLAST algorithm was used to query BAC clones against the nt and EST databases to search for evidence of expression of candidate genes from the MYP2 interval. Exon/intron boundaries were also determined from known gene structures described in the NCBI Map Viewer. Reverse-transcription polymerase chain reactions (RT-PCR) and queries of the UniGene database were performed to substantiate gene expression in ocular tissues. Coding regions, intron/exon boundaries and untranslated exons of all known genes were sequenced using genomic DNA samples from 12 affected and 8 unaffected MYP2 pedigree members, and from 4 unaffected and 1 highly myopic external controls. Polymorphic sites were compared to known variants from the dbSNP database. 116 polymorphisms were found by direct sequencing; 12 were missense, 17 were silent, 34 were not translated, 51 were intronic, and 2 were homozygous deletions. Forty-six polymorphisms were novel. Novel SNPs were submitted to dbSNP; observed frequencies were submitted for known SNPs. No identified sequence alterations were associated with the disease phenotype. Further studies of MYP2 candidate genes, including analysis of putative genes predicted in silico, are underway to ascertain the determinant(s).

A mutation creating an upstream initiation codon in the SOX9 5 UTR causes campomelic dysplasia. *G. Scherer*¹, *R. Pop*¹, *D. Johnson*², *J. Tolmie*², *D. Morris-Rosendahl*¹. 1) Institute of Human Genetics, Freiburg, Germany; 2) Institute of Medical Genetics, Yorkhill NHS Trust, Glasgow, UK.

Campomelic dysplasia (CD) is a semilethal skeletal malformation syndrome that results from de novo heterozygous mutations in SOX9. These mutations are distributed over the entire coding region and cause loss-of-function of the protein, resulting in haploinsufficiency. We report on a 2 year-old girl with a 46,XX karyotype who has clinical and radiological features of surviving CD, including micrognathia, tracheomalacia, small scapulae, 11 pairs of ribs and short ischia. Sequence analysis of the SOX9 coding region failed to reveal a mutation. However, a heterozygous mutation G>A at position 188 in the 5 UTR was found. The patients mother, father and healthy brother were homozygous G/G at this position, as were 100 control chromosomes. Paternity was confirmed by microsatellite marker analysis. The G>A mutation creates an upstream translation start codon (GTG>ATG) with a much better fit of its flanking sequence to the Kozak consensus than that at the wildtype ATG start codon (4/6 vs. 1/6). If used, the upstream ATG would lead to translation of a short peptide of 62 amino acids from a reading frame that terminates just after the wildtype ATG start codon. Such an upstream open reading frame could reduce or prevent translation of the wildtype protein. Reduced amounts of SOX9 protein from the mutant mRNA would be compatible with the milder phenotype of the patient. Although described only rarely in human genetic disease and for the first time here for CD, mutations creating upstream ATG codons may be more common than generally assumed.

Canine X-linked ectodermal dysplasia as a model for the human disease: mutational analysis and further characterization of disease. *M.L. Casal¹, E.A. Mauldin², O. Gaide³, J.L. Scheidt¹, J.L. Rhodes¹*. 1) Medical Genetics, Veterinary Hospital, Univ Pennsylvania, Philadelphia, PA; 2) Dept Pathobiology, Univ Pennsylvania, Philadelphia, PA; 3) Dept Dermatology, Univ Hospital of Geneva, Switzerland.

A breeding colony of dogs with X-linked ectodermal dysplasia (XHED) is maintained at the University of Pennsylvania for the study of both mechanisms of disease and similarities to human XHED. We sequenced the canine ED1 gene and determined the disease to be caused by a nucleotide substitution (G to A) in the splice acceptor site of intron 8. A cryptic acceptor site is subsequently used within exon 9, resulting in a truncated message and, thus, the loss of the TNF homology domain, the receptor-binding site of ectodysplasin. As in human patients with XHED, affected dogs are at risk for pulmonary disease characterized by frequent infections and unexpected deaths due to opportunistic respiratory infections. Previous experiments aimed at examining the XHED dogs immune system showed no differences between affected and normal dogs. Recently, we examined morphological differences in respiratory organs from XHED dogs and normal age-matched controls to understand the basis of respiratory disease. Histopathologic changes consisted of complete loss of branched tubuloalveolar mucous glands from nasal turbinates, trachea, and bronchi resulting in reduced respiratory ciliary motility. In humans, several reports point to a total absence of mucous glands in pharynx, larynx, trachea, and large and small bronchi, sometimes associated with complete tracheal obstruction by mucous debris. This finding was thought to be the basis for the observed abnormal tracheobronchial secretions and increase in susceptibility to respiratory infections. Other canine organs with glandular components were examined; there was complete absence of eccrine glands from the gastrointestinal tract and from footpads, which is the only site of sweat glands in normal dogs. In humans, mucous glands are absent in the upper esophagus and hypoplastic in the colon. This is thought to predispose affected children to intussusceptions that may also have a fatal outcome.

***DNALI* and *NME5*: two candidate genes for Kartagener syndrome with ultrastructurally normal cilia.** M.L. Lacombe¹, A. Moore^{2,3}, A. Munier¹, A. Coste^{2,4}, N. Desmazes-Dufeu⁴, B. Duriez³, S. Amselem^{3,4}, E. Escudier^{2,4}. 1) INSERM U402, Paris; 2) INSERM U492, Creteil; 3) INSERM U468, Creteil; 4) AP-HP, Paris; France.

Primary Ciliary Dyskinesia (PCD) is a rare disease characterized by airway infections (bronchiectasis and rhinosinusitis) due to abnormal ciliary structure and function mainly due to defects of dynein arms. In half cases, respiratory symptoms are associated with *situs inversus* therefore defining Kartagener syndrome (KS). In our population, 19% of patients with KS display cilia with normal axonemal ultrastructure. In these cases, diagnosis of PCD is based on typical clinical features and immotile cilia. As no structural defect could be identified by electronic microscopy, we postulated that genes responsible for the disease could encode small structural or enzymatic proteins important for ciliary beating. We selected two genes: *DNALI*, a gene orthologous to *lc1* identified in *Chlamydomonas reinhardtii*, coding for a dynein light chain of the outer dynein arm (ODA); and *NME5* encoding an axonemal protein, Nm23-H5 homologous to nucleotide diphosphate kinases. Lc1 (22kDa) belongs to the leu-rich family protein. This protein binds at the ATP hydrolytic site (*i.e.* motor domain) of the ODA, permitting the binding with the adjacent B-microtubule, an interaction that plays a key role in microtubule sliding. In silico analysis revealed the existence of a potential human orthologous sequence on chromosome 14q24.3. This sequence designated *DNALI* (standing for dynein axonemal light chain 1) contains 8 putative exons with corresponding ESTs found in human ciliated tissues. The predicted protein, which is composed of 151AA, contains a leu-rich repeat domain. *NME5* was selected because of its expression in tissues with axonemal structures, *e.g.* trachea and testis. This gene (5q23-31) encodes a kinase (24.2 kDa) associated with central microtubules and outer doublets. We screened for mutations these two genes in our patients with KS and normal cilia. Although no mutations have been so far identified, the location and the function of the corresponding proteins make those two genes good candidates to be investigated in patients with KS characterized by immotile cilia and absence of axonemal defects.

Assessing the possible utilization of CFTR-M470V genotyping for CF risk determination. *P.F. Pignatti¹, F. Pompei², A. Bonizzato³, A. Beghini¹, B.M. Ciminelli², C. Bombieri¹, C. Ciccacci², C. Castellani³, G. Modiano².* 1) Sec. Biology and Genetics, Dpt. Mother & Child/Biol & Gen, Univ Verona, Verona; 2) Dpt. of Biology, University of Roma-Tor Vergata, Roma; 3) Veneto Regional CF Center, Hospital of Verona, Verona; Italy.

In a previous study on European non-CF individuals, we found that random CFTR gene mutations were preferentially present in the genes carrying the M allele of the M470V common polymorphism (unpublished data). Therefore, we hypothesized that CFTR alleles with the M variant should have an increased risk of carrying a CF-causing mutation. We collected 177 nuclear families (parents and a CF child) for a total of 531 subjects, selected for the presence of at least one non-DF508 mutation in the genotype of the CF child, after the screening of the most common CF mutations in the population. The haplotypes formed by the M470V polymorphism and the CF causing mutation have been established in 152 families. We had 100 DF508 alleles, 172 alleles with one non-DF508 common mutation, 32 alleles with an unidentified mutation. Giving the known association between DF508 and M allele, we focused our analysis on the 204 CF-non-DF508 alleles: 171 (84%) of these genes carried the M allele. Frequency of the M allele in the normal population is 38.5% (Hum Genet 106:172,2000), the difference is significant ($p < 0.00001$). These results confirm for the CF alleles a preferential association with the M allele, so that the risk for an MM or MV individual to be heterozygote for a CF mutation is significantly higher than for a VV subject (4.4% and 2.4% respectively, vs 0.5%). Accordingly, the risk to have a CF child increases for the couple in which both parents have at least one M allele (e.g. 0.028% for a VVxVV couple, 0.19% for a MMxMM couple, both negative for the DF508). Therefore, typing a subject for the M470V polymorphism can reduce or increase his/her risk to be heterozygote for a CF mutation, so that further analysis could be suggested only for individuals (or couples) with an increased risk. Acknowledgments: Italian CF Foundation.

DUCHENNE muscular dystrophy caused by a rare mid-intronic substitution activating a cryptic exon in the DMD gene. *N. Lannoy¹, I. Abinet¹, P. Van den Bergh², Ch. Verellen-Dumoulin¹.* 1) Dept Genetics, UCL, Saint-Luc Hosp, Brussels, Belgium; 2) Departement of Neurology, UCL, Brussels, Belgium.

The defects in the Duchenne muscular dystrophy (DMD) gene are mainly exon deletions (60%), duplications (10%) and small mutations (30%). The precise identification of the mutation in each patient is necessary to offer accurate and safe genetic counseling, prenatal diagnosis and future gene therapy. In this context, eight different mutations have been identified in 7 DMD and in 1 BMD/XLCMD patients using Protein Truncated Test (PTT), DGGE and sequencing methods. Six mutations have not been reported previously : two nonsense mutations (C569X and L654X), one frameshifting deletion (6438delG), two splicing defects (6614+1G>A, 10086+1G>A) and one mid-intronic substitution (1812+601A>G). The latter mutation was described in a family where two brothers were diagnosed with DMD at 3 years and 18 months of age after clinical examination and muscle biopsy immunofluorescence analysis. The whole dystrophin genomic gene was tested by multiplex PCR and Southern blotting without finding a gross gene defect. Mutation search was performed by PTT on total muscle RNA from the eldest brother. Aberrant mobility on PTT gel was identified from c-DNA covering exons 9 to 18. Curiously, no mutation has been found when sequencing analysis was performed on genomic exons 9 to 18 with their adjacent intronic sequences. An elongated RT-PCR fragment from this region was found on acrylamide gel . Its sequence showed a non in-frame extraneous sequence of 121 pb intercalated between exons 15 and 16. An in silico search of the whole dystrophin genomic sequence revealed that this insert corresponds to a cryptic exon flanked by consensus splice sites located in the mid-part of intron 15 due to a single intronic substitution. This mutation is also present in the brother's and mothers DNA but absent in a control population. Mid-intronic mutation may be pathogenic. At this time, the incidence of this type of mutation in the DMD gene is unknown but a preliminary proportion of 6% has been recently proposed among the non-deleted/non-duplicated cases.

SBDS Localisation and Effects of Gene Mutation on Protein Expression. *N. Richards*¹, *G.R.B. Boocock*^{1, 2}, *J.M. Rommens*^{1,2}. 1) Program in Genetics & Genomic Biology, Hospital for Sick Children, Toronto, Ontario, Canada; 2) Department of Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, Canada.

Genetic studies have led to the identification of mutations that lead to Shwachman-Diamond syndrome (SDS) in an uncharacterised gene named *SBDS*. *SBDS* is broadly expressed and encodes a polypeptide of 250 amino acid residues with a predicted molecular mass of 28.8 kDa. Immunofluorescence microscopy of SV40-transformed fibroblasts indicated diffuse localisation throughout cells with heterologous expression of HA, *c-myc* and GFP-*SBDS* fusion polypeptides. Rabbit polyclonal antibodies have been generated to directly assess endogenous *SBDS*; one antibody was raised against recombinant protein, and two others were raised against short synthetic peptides derived from the amino and carboxyl portions. The *SBDS* antibodies detected the epitope-tagged *SBDS* in transfected fibroblasts as well as an endogenous 30 kDa band in extracts from various cell lines by western blotting. In order to assess the effects of *SBDS* mutations on expression, proteins were extracted from EBV-transformed B-lymphoblastoid cell lines derived from unrelated SDS patients and control individuals. Common mutations account for the alleles seen in more than 50% of SDS patients and corresponding cell extracts exhibited no detectable levels of the 30 kDa protein. Extracts from a patient heterozygous for a common and a rare splicing mutation [258+1G>C], also revealed no detectable protein. Patients heterozygous with rare missense mutations [97A>G] (K33E), [505C>T] (R169C) and [506G>A] (R169H) did indicate detectable levels of full-length protein. Five patients meeting diagnostic criteria, but carrying no known mutations to date, expressed levels of *SBDS* protein comparable to controls. Given that most SDS patients do not exhibit detectable levels of protein, decreased *SBDS* expression contributes to the SDS phenotype.

Expression analysis of genes from the Williams-Beuren syndrome region at 7q11.23. *W. Loo*¹, *L.R. Osborne*^{1,2}. 1) Molecular & Medical Genetics, University of Toronto, Toronto, Ontario, Canada; 2) Medicine, University of Toronto, Toronto, Ontario, Canada.

Williams-Beuren syndrome (WBS) is caused by the deletion of more than 20 genes on chromosome 7. As a first step towards identifying dosage sensitive genes that might be more likely to cause a phenotypic effect, we analyzed the expression levels of candidate genes in transformed human lymphoblast cell lines from families with WBS. By analysis of individuals with different rearrangements of 7q11.23 we hope to correlate gene expression with the phenotypic features seen in patients with WBS. Real-time RT-PCR was used to measure the transcript level of candidate genes relative to three control genes, *-actin*, *STARD7* and *HPRT*. The ratio of test to control transcript in each proband was then compared to that of first-degree relatives.

We found that the level of *WBSCR16* (outside the deletion) was not altered in individuals with a deletion or inversion, but was increased in an individual with a duplication of 7q11.23. Surprisingly, the level of *GTF2IRD1* transcript (within the common deletion) showed no difference in any of these individuals. *GTF2I* expression, however, was reduced on average to 55% in 10 WBS deletion patients (range 42%-70%) and increased to 180% in the duplication patient. No difference in expression level was seen in three parents carrying an inversion. Analysis of these genes in two previously reported individuals with WBS inversions and clinical features, did not reveal any differences in expression.

These results suggest that the WBS inversion polymorphism does not affect expression of *GTF2I*, *GTF2IRD1* or *WBSCR16*. Furthermore, it is unlikely that these genes play a role in the clinical symptoms seen in atypical WBS inversion patients. The hemizygous deletion of *GTF2I*, but not *GTF2IRD1*, however, affects the level of gene expression, at least in lymphoblasts, and suggests that *GTF2I* may be haploinsufficient in WBS. Further studies of individuals with other rearrangements of 7q11.23 and of mice with deletions of these genes will help to clarify their involvement in this syndrome.

CHARACTERISATION OF GAA REPEAT-CONTAINING FRIEDREICH ATAXIA YAC TRANSGENIC MICE: RESCUE OF THE FRATAXIN KNOCKOUT. *S. AL-MAHDAWI¹, F. YADOLLAHI¹, A. OBISESAN¹, Z. WEBSTER², H. PUCCIO³, M. KOENIG³, M.A. POOK¹.* 1) MEDICAL GENETICS, IMPERIAL COLLEGE LONDON, HARROW, UK; 2) ES CELL FACILITY, MRC CSC, HAMMERSMITH HOSPITAL, UK; 3) IGBMC, CNRS/INSERM UNIVERSITE LOUIS PASTEUR, ILLKIRCH, FRANCE.

Friedreich ataxia (FRDA) is mainly caused by a homozygous GAA repeat expansion mutation within intron 1 of the FRDA gene, resulting in reduced frataxin expression. To establish an FRDA GAA repeat mouse model, we have generated two lines of human FRDA YAC transgenic mice that contain GAA repeat expansions within the appropriate genomic context: YG8 contains two GAA sequences of 190 and 90 repeats, while YG22 contains a single 190 repeat sequence. Intergenerational instability and age-related somatic instability of the GAA repeat has been detected in both lines, with the most pronounced somatic instability arising from CNS tissues. We now show that both of the human FRDA YAC transgenic lines can successfully rescue the embryonic lethality of homozygous frataxin knockout mice. The levels of expression of frataxin mRNA and protein in different somatic tissues from the transgenic and rescued mice have been determined by RT-PCR and western blot analysis. The expression levels have then been compared with the degree of GAA repeat instability. Further histological, biochemical, metabolic and functional studies are currently underway to determine the effectiveness of these mice as a GAA-repeat based model for FRDA.

Characterization of Bardet-Biedl syndrome 2 (Bbs2) knockout mice indicate a defect in intracellular transport.

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Bardet-Biedl syndrome (BBS) is a heterogeneous, pleiotropic human disorder characterized by obesity, retinopathy, polydactyly, renal and cardiac malformations, learning disabilities, hypogenitalism, and an increased incidence of diabetes and hypertension. It has been suggested that the BBS phenotypes are the result of defective cilia formation or function. We show that mice lacking the Bbs2 protein have major components of the human phenotype including obesity. In addition, these mice have phenotypes associated with cilia dysfunction including retinopathy, renal cysts and male infertility. With the exception of male infertility, these phenotypes are not due to a general defect in cilia formation. We demonstrate that BBS2 retinopathy involves apoptotic death of photoreceptors, the primary ciliated cells of the retina. Photoreceptor cell death is associated with mislocalization of rhodopsin indicating a primary defect in transport. Finally, we demonstrate that Bbs2 heterozygous mice become obese suggesting that genes involved in intracellular transport are candidates for non-syndromic human phenotypes such as obesity.

Transgenic mice expressing Y99C-GCAP1-EGFP exhibit a cone dystrophy phenotype. *n. roychowdhury*¹, *k. zhang*¹, *y. sauve*¹, *j. frederick*¹, *w. baehr*^{1,2,3}. 1) Ophthalmology, Moran Eye Center, Salt Lake City, UT; 2) Department of Neurobiology and Anatomy, University of Utah Health Science Center, Salt Lake City, Utah 84112; 3) Department of Biology, University of Utah, Salt Lake City, UT 84112.

GCAP1, a Ca²⁺-binding protein of the calmodulin gene family with four EF hand motifs, is responsible for regulation of guanylate cyclase (GC) in photoreceptors, and production of cyclic GMP (cGMP), the internal transmitter of phototransduction. In the dark at high [Ca²⁺]_{free}, Ca²⁺-bound GCAP1 is inactive, but in light when [Ca²⁺]_{free} is reduced due to closure of cGMP-gated channels, Ca²⁺ dissociates from GCAP1, an event which turns GCAP1 into a GC-activator. A Y99C mutation in EF3 of GCAP1 was linked to autosomal dominant cone dystrophy in a British pedigree. To investigate the consequences of the mutant GCAP1 expression in the living retina, we generated mice expressing a transgene consisting of a 5 kb upstream region, the entire GCAP1 gene including all introns and the polyadenylation site, the Y99C mutation in exon 3, and a EGFP fused to the C-terminal of GCAP1 (Y99C-GCAP1-EGFP). As monitored by EGFP intrinsic fluorescence, the transgene was expressed in both rods and cones in a line that expressed wild-type and mutant GCAP1 at a ratio of (1:1 1:2). At 6 months of age, the rod ERG response in transgenic mice was normal, while the cone response showed reduced cone b-waves, and reduced cone flicker amplitudes, consistent with cone dystrophy. These results show that the phenotype of transgenic mice expressing Y99C-GCAP1-EGFP closely resembles the cone dystrophy observed in human patients.

The molecular basis of a new class of ABCR mutations in patients with Stargardt disease (STGD). W.

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ABCR (ABCA4) is a photoreceptor-specific member of the ATP-binding cassette (ABC) family, and mutations of ABCR are associated with many retinal phenotypes including Stargardt disease, Fundus Flavimaculatus, combined cone-rod dystrophy and retinitis pigmentosa. Our purpose is to develop an experimental system for investigating mechanisms regulating intracellular trafficking of ABCR and for testing mutations found in domains involved in cellular localization of ABCR. Our approach is to express a human ABCR transgene in photoreceptors of *Xenopus laevis* tadpoles. DNA fragments containing both wild type and mutated (L541P, R602W and C1490Y) ABCR coding sequence were cloned into the pXOP vector containing the promoter for the *X. laevis* rhodopsin gene, and used for transgenesis. The retinas of 2-4 week old tadpoles were studied for the expression and localization of transgenic ABCR by immunofluorescence methods. We found that expression was restricted to photoreceptor cells and was variable from cell to cell. In some cells very bright staining of the rims of the discs was observed in rod outer segments (ROS). We found that some pathogenic mutations cause mislocalization of ABCR with stacking of misfolded protein in the rod inner segment. We conclude that human ABCR can be expressed from a transgene in *Xenopus* photoreceptors with appropriate localization to the ROS. However, we have identified a new class of ABCR mutations, which lead to improper localization, thus rendering a null effect. Mutations responsible for this effect are found with other mutated alleles in patients with retinal degenerative phenotypes.

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Risk factors of intermediate allele instability for the fragile X CGG repeat. *S. Nolin, G. Houck, A. Glicksman, S. Li, J. Silverman, W.T. Brown.* Human Genetics Dept, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY.

Fragile X alleles with ~40 to ~60 repeats are both interesting and challenging from a diagnostic and genetic counseling perspective because of the difficulty in predicting instability. Several laboratories have shown that alleles in this repeat range may be stably inherited, expand or contract. We examined 5 risk factors that may influence instability: 1) repeat size, 2) AC polymorphisms flanking the repeat, 3) CGG repeat structure, 4) length of 3' pure repeats, and 5) parental origin of transmission. While no specific AC haplotypes were associated with instability, the larger intermediate alleles were associated with greater instability. The 3' length of CGGs without AGG interruptions was found to be a risk factor for instability; 14% of transmissions with 34-40 pure CGGs and 43% with >40 pure CGGs were unstable. We found a similar proportion of unstable maternal and paternal transmissions among families with intermediate alleles. In the 40-49 repeat sizes, 10% (4/39) unstable maternal transmissions and 13% (3/22) paternal transmissions were observed. In the 50-60 repeat sizes, 19% (10/52) unstable maternal and 18% (2/11) paternal transmissions were observed. The combined mutation rate of repeats from 40-60 was 15% for maternal or paternal transmissions. These observations are markedly different from those of Sullivan et al. (AJHG 70:1532, 2002) who observed a higher mutation rate of among paternally as compared to maternally transmitted alleles. Most strikingly, none of the families with intermediate alleles had transmission patterns resembling the large expansions observed in maternal transmissions of fragile X premutation females.

Bartter Syndrome Type II associated with a Novel Mutation in KCNJ1. A. Jeong¹, M. Merideth¹, M. Freundlich², W.A. Gahl¹, R. Kleta¹. 1) SHBG, MGB, NHGRI, NIH, Bethesda, MD; 2) Department of Pediatrics, University of Miami, Miami, FL.

Bartter syndrome type II is an autosomal recessive renal salt losing nephropathy caused by mutations in KCNJ1. Five alternatively spliced variants code for 3 different isoforms. Transcript variant 2 (also named ROM-K2), derived from exons 1 and 5 of KCNJ1, is expressed in the kidney and codes for a 372 amino acid protein. Mutations in SLC12A1 (Na²ClK cotransporter), CLCNKB (chloride channel), and BSND (chloride channel subunit) lead to Bartter syndromes type I, III, and IV, respectively. Here we report the clinical and molecular findings in a boy with renal salt wasting, neonatal hyperkalemia and subsequent hypokalemia. The patient was born at 28 weeks' gestation with a birth weight of 1402 g and a history of polyhydramnios. His same sex twin was born without maternal polyhydramnios and had an uneventful clinical course. Initially, a diagnosis of twin-twin transfusion syndrome was considered. Postnatally, a dichorionic, diamniotic, non-fused twin placenta was documented. Within 72 hours after birth the affected twin displayed marked hyperkalemia (6-7 mmol/l), hyponatremia (129 mmol/l), azotemia (BUN 44 mg/dl), natriuresis (urine Na 118 mmol/l) and polyuric renal failure (creatinine 2.0 mg/dl). The hyperkalemia gradually changed to hypokalemia (3 mmol/l). At age 6 months all blood chemistries were normal without any supplements or medications. At age 3 years the patient presented with polyuria, polydypsia, growth retardation, hypoosmolar urine, elevated prostaglandins, metabolic alkalosis, hypercalciuria and nephrocalcinosis. Treatment with potassium supplementation and the COX-2 inhibitor, rofecoxib, corrected all laboratory abnormalities and improved the anthropometric delay. Genomic DNA analysis of KCNJ1 disclosed that the patient was compound heterozygous for two missense mutations, a known R311W and a novel L359R mutation. In summary, we present a rare but important cause of neonatal salt losing tubulopathy with initial non-oliguric hyperkalemia, eventually evolving into a polyuria-polydypsia hypokalemic phenotype accompanied by hypercalciuric nephrocalcinosis.

A second locus for glaucoma associated with pigment dispersion maps to chromosome 18q22. *E.A. DelBono¹, D.S. Greenfield², R.K. Parrish², L.M. Olson³, J.L. Haines³, J.L. Wiggs¹.* 1) Dept Ophthalmology, Harvard Medical School/MEEI, Boston, MA; 2) Bascom Palmer Eye Institute, Miami, FL; 3) Center for Human Genetics, Vanderbilt University School of Medicine, Nashville, TN.

Glaucoma associated with pigment dispersion (GPDS) is the most common form of glaucoma affecting the young adult population. This disease is characterized by release of pigment granules from the iris that are distributed throughout the eye (pigment dispersion). Approximately 50% of individuals affected with pigment dispersion develop glaucoma associated with elevated intraocular pressure and degeneration of the optic nerve. In humans, GPDS is inherited as an autosomal dominant trait, while similar syndromes in mice exhibit more complex inheritance patterns. We have previously mapped one locus for this condition to chromosome 7q36, and have also identified pedigrees that do not demonstrate linkage to this region indicating genetic heterogeneity. The largest family not linked to the 7q36 region was used for a second genome-wide scan. Fifty-one family members (13 affected) were genotyped for two hundred thirty eight microsatellite repeat markers spanning the human genome at approximately 10 cM intervals. A maximum two-point lod score of 4.2 was found for marker D18S1127. Haplotype analysis identified a 13 cM (12 Mb) region extending from D18S484 to D18S483. Three smaller families also have consistent segregation of haplotypes using microsatellite markers located in this region. These results provide evidence for a new locus responsible for glaucoma associated with pigment dispersion on chromosome 18q22. Candidate genes located within this region with ocular expression are currently being screened for causative mutations.

BBS8 analysis in a cohort of 119 Bardet-Biedl families: a single multiplex homozygous family with partial triallelism. *H. Dollfus¹, C. Stoetzel², V. Laurier², D. Bonneau³, A. Verloes⁴, L. Faivre⁵, F. Perrin-Schmitt², M. Cossée⁶, J.L. Mandel⁶, the French BBS group⁷.* 1) Service de Genetique Medicale, Hopitaux Univ de Strasbourg, Strasbourg, France; 2) Laboratoire de gntique, Facult de Mdecine, Hpitaux Universitaires de Strasbourg. Strasbourg. France; 3) Service de gntique, CHU Angers. France; 4) Unit de gntique clinique, Hpital Robert-Debr, Paris. France; 5) Service de gntique, CHU Dijon, Dijon, France; 6) Laboratoire de diagnostic gntiqueCHRU,Strasbourg, France; 7) France.

Introduction : Bardet-Biedl syndrome (BBS) is a genetically heterogenous condition characterized by retinitis pigmentosa, obesity, polydactyly, renal and genital malformations and learning disabilities. To date 7 genes have been identified and triallelic inheritance demonstrated for some families. In 2003, the identification of BBS8 (TTC8) , a centrosomal and basal body protein, designated BBS as a ciliary dysfunction disorder, moreover, one patient had also situs inversus (Ansley et al, 2003). A French cohort of BBS families is currently being explored for all the BBS genes. We present herein our results for BBS8. **Families and methods:** BBS8, mapped in the 14q32.11 region and spanning 15 exons, was analyzed by DHPLC combined to direct sequencing, for 119 families. Two sporadic cases presented with a BBS phenotype plus situs inversus. **Results:** Only one consanguineous family, with three affected siblings carried a homozygous splice-site mutation at the level of the exon 4 of BBS8. Interestingly, an additional splicing mutation was detected in BBS7 from one affected child but did not cosegregate with the disorder as the two other affected patients did not carry this third allele, excluding for BBS8 and BBS7 the triallelic model in this family. To date no other mutation in BBS genes has been detected for this family. The two BBS patients with situs inversus were not found to carry a BBS8 mutation. **Conclusions:** Mutations in BBS8 are very rare in the French cohort as for a previously published cohort. It could be expected that less than 1% of the BBS families carry BBS8 mutations. The phenotype BBS plus situs inversus is not related to BBS8 mutations for our patients.

Wnt pathway gene expression changes associated with retinal degeneration in the rd1 mouse. *A.S. Hackam¹, D.J. Nolan¹, R. Strom², D. Liu², J. Qian², D. Otteson², T. Gunatilaka², R.H. Farkas², I. Chowers², M. Kageyama³, P. Campochiaro², G. Parmigiani², D.J. Zack².* 1) Bascom Palmer Eye Institute, University of Miami, Miami, FL; 2) Johns Hopkins University School of Medicine, Baltimore, MD; 3) Santen Pharmaceutical, Takayama, Japan.

Mutations in over 100 genes have been implicated in retinal degenerations. To understand the molecular events contributing to photoreceptor death and identify novel neuronal protection factors we have used custom retinal microarrays to analyze the rd1 mouse, a well-characterized model of human retinal degeneration. The custom array contains genes involved in normal retinal function, development, and disease. Gene expression in rd1 retinas was compared with age-matched controls at three time-points: peak of rod photoreceptor degeneration (Day (D) 14), and during early (D35) and late (D50) cone degeneration. The microarrays showed limited overlap of the genes with increased expression, suggesting the involvement of temporally distinct molecular pathways during rod and cone death. Increased expression of genes involved in cell proliferation pathways and oxidative stress was observed at each time-point. Additionally, the microarrays, confirmed by quantitative PCR, demonstrated marked differential expression of mediators of the Wnt signaling pathway, a multigene pathway with well-described roles in embryonic development and oncogenesis. Although Wnt signaling is anti-apoptotic in certain situations, its function during retinal degeneration has not been explored. The Wnt-related gene, Dickkopf 3 (Dkk3), which was upregulated during rod and cone death, was investigated further. We localized Dkk3 transcripts to the inner nuclear layer of the retina, potentially to Muller glia, the cells involved in photoreceptor protection in other retina damage situations. To determine the activity of Dkk3, HEK293 cell lines stably expressing Dkk3 were created. Cell death assays demonstrated that Dkk3 had a significant pro-survival effect on cells exposed to oxidative stress. These results suggest a hypothesis in which Dkk3 and Wnt signaling are activated in the retina, potentially as part of the glial response to photoreceptor injury.

LEBER CONGENITAL AMAUROSIS: COMPREHENSIVE SURVEY OF THE GENETIC HETEROGENEITY, REFINEMENT OF THE CLINICAL DEFINITION AND PHENOTYPE-GENOTYPE CORRELATIONS AS A STRATEGY FOR MOLECULAR DIAGNOSIS. *S. Hanein, I. Perrault, S. Gerber, A. Munnich, JM. Rozet, J. Kaplan.* INSERM U393, Hopital des Enfants Malades, Paris, France.

Leber congenital amaurosis (LCA) is the earliest and most severe form of all inherited retinal dystrophies, responsible for congenital blindness. Disease mutations have been hitherto reported in seven genes. These genes are all expressed preferentially in the photoreceptor cells or the retinal pigment epithelium but they are involved in strikingly different physiologic pathways resulting in an unforeseeable physiopathologic variety. This wide genetic and physiologic heterogeneity that could largely increase in the coming years, hinders the molecular diagnosis in LCA patients. The aim of the present work was to establish phenotype-genotype correlations as a pre-requisite to genotyping of patients affected with LCA. A comprehensive mutational analysis of the all known genes was performed using DHPLC and direct sequencing in 179 unrelated LCA patients, including 52 familial and 127 sporadic (27/127 consanguineous) cases. To establish phenotype-genotype correlations, the clinical history of all patients with mutations was carefully revisited in search for phenotype variations. Mutations were identified in 47.5% patients. GUCY2D appeared to account for most LCA cases of our series (21.2%), followed by CRB1 (10%), RPE65 (6.1%), RPGRIP1 (4.5%), AIPL1 (3.4%), TULP1 (1.7%) and CRX (0.6%). Sound genotype-phenotype correlations were found that allowed to divide patients into two main groups. The first one includes patients whose symptoms fit the traditional definition of LCA i.e. congenital or very early cone-rod dystrophy, while the second group gathers patients affected with severe yet progressive rod-cone dystrophy. Besides objective ophthalmologic data allowed to subdivide each group into two subtypes. Based on these findings, we have drawn decisional flowcharts directing the molecular analysis of LCA genes in a given case. This work which lightens henceforward the heavy task of large scale genotyping in LCA and makes possible to establish genetically defined subgroups of patients ready for therapy.

Human ARR-CHIP: A high throughput sequencing array for genotyping patients with autosomal recessive retinitis pigmentosa (ARRP). *M.N. Mandal¹, J.R. Heckenlively¹, L. Chen¹, T. Burch¹, P.A. Sieving², R. Ayyagari¹.* 1) Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI; 2) NEI/NIDCD, National Institutes of Health, Bethesda, MD.

Purpose: Retinitis Pigmentosa (RP) is a group of inherited diseases that cause degeneration of the retina leading to blindness. RP is transmitted in autosomal dominant, autosomal recessive and X-linked manners and these are genetically heterogeneous disorders. For recessive RP alone there are more than 14 known genes comprising more than 30 kb sequence. Screening all these candidate genes for mutations is expensive and time consuming. We have designed and developed sequencing arrays which can successfully screen exons and flanking intronic sequences of most of eleven recessive RP genes in a cost and time efficient manner. **Results:** The ARR-CHIP contains oligonucleotide sequencing probes that are tiled using standard Affymetrix protocol. Both strands of the coding sequence of ARR-CHIP genes are arrayed on the ARR-CHIP along with appropriate control probes. We use genomic DNA to amplify the exonic regions and amplicons are pooled, fragmented, hybridized and scanned. We have sequenced 25 samples (>665 kb sequence) using these ARR-CHIPs. This analysis was observed to assign correct base call at 97.27% of nucleotide positions. Replicate experiments demonstrated > 99% reproducibility. Approximately 20 sequence changes were detected (15-24) in each sample (30 kb) and 90% of these sequence changes matched with known SNPs, indirectly validating the performance of these chips. ABI sequencing of these samples validated the sequence changes detected by the chip in almost every cases with a few exception of false positives. These false positive calls came from regions of higher G-C or due to low hybridization signal. We found a few novel mutations in some families and alterations in more than one genes in few samples. **Conclusions:** ARR-CHIP is a high-throughput sequencing tool for reliable identification of mutations in patients with ARR-CHIP in a single assay. These chips also provide an opportunity to determine complex phenotypes, gene interactions and modifiers involving more than one gene arrayed on the chips.

Two different retinal dystrophies accounted for by different CRB1 mutations in a single nuclear family. I.

Perrault, S. Hanein, S. Gerber, A. Munnich, JM. Rozet, J. Kaplan. Dept Genetics, INSERM U393, Paris, Cedex, France.

The aim of this study was to identify the molecular bases of the occurrence of late onset retinitis pigmentosa (RP) and Leber congenital amaurosis (LCA) in a single small nuclear family. Leber congenital amaurosis was unambiguously diagnosed in the third child of a non consanguineous family of French descent. In the first months of life he was found to carry typical signs of the disease ie absence of ocular pursuit, searching nystagmus, oculo-digital signs of Franceschetti and non recordable ERG. At birth, his parents were considered healthy. Three years later, an ophthalmologic examination of the father, requested by a company doctor, detected a moderate visual loss. Complete explorations of this 38 year old man revealed typical retinitis pigmentosa. The screening of the seven already known LCA genes was first undertaken in the LCA patient using DHPLC and direct sequencing and then extended to the whole family. The LCA Patient was found to be compound heterozygote for two CRB1 mutations (p.L1107P/p.S1025I). Segregation analysis showed that each of the two mutations was inherited from one parent. The discovery of RP in the father prompted us to screen the whole CRB1 sequence. Interestingly, two other non conservative amino-acid changes were identified in the father affected with RP(V1325F, A1328V). Subsequently, the segregation analysis showed that the father carried two mutant CRB1 alleles: L1107P, inherited from his own healthy mother and transmitted to his son affected with LCA, and a complex mutant allele [V1235F and A1328V] inherited from his own healthy father. This observation emphasises that CRB1 mutations can be responsible for late-onset RP or LCA depending on the association of disease alleles in the patient. Considering that the patients in the reported family shared 50% of their genetic background, one can reasonably speculate that genotype-phenotype correlations may exist for the CRB1 gene.

Novel disease causing variations in PITX2 and CYP1B1 in secondary congenital glaucoma patients with anterior segment dysgenesis (ASD). *B. Rautenstrauss*¹, *G. Chavarria*¹, *C. Mardin*², *A. Reis*¹. 1) Institute of Human Genetics, Friedrich-Alexander-University Erlangen-Nuremberg, Germany; 2) Department of Ophthalmology, Friedrich-Alexander-University Erlangen-Nuremberg, Germany.

Glaucomas are a clinically and genetically heterogeneous group of optic neuropathies. The disease affects over 40 million people worldwide and is the second most prevalent cause of bilateral blindness in the Western world. In contrast to primary glaucomas the secondary congenital glaucomas are associated with a variety of anomalies involving different tissues of the eye summarized as anterior segment dysgenesis (ASD). Distinct subtypes of ASD include aniridia, Axenfelds anomaly, Riegers anomaly, iridogoniodysgenesis, Peters anomaly and posterior embryotoxon. Mutations in the cytochrome P450 1B1 (CYP1B1) gene are frequently found in primary congenital glaucoma (PCG) patients but have also been reported for Peters anomaly. Mutations in the transcription factors FOXC1, PITX2, and PAX6 cause different kinds of ASD. We analysed 11 Riegers anomaly patients, 11 Peters anomaly patients and 48 controls for mutations in the FOXC1, PAX6, PITX2 and CYP1B1 genes. Six CYP1B1 SNPs associated with PCG were observed with similar frequencies in ASD patients and controls. For PAX6 and FOXC1 we found intronic and exonic benign variations. For PITX2 one Riegers anomaly patient was heterozygous for a novel Ala187Thr aminoacid exchange. This novel variation is located in an evolutionary highly conserved position of PITX2. Furthermore two sporadic Riegers anomaly patients with compound heterozygous mutations in the CYP1B1 gene were identified. One patient presented the mutations Trp57Stop and Leu343del. The Trp57Stop truncates the protein by 486 aminoacids. The newly described Leucin deletion eliminates one out of three leucines, located in the highly conserved I-helix region of the protein. The second patient carries also a Trp57Stop mutation and additionally the c.1210dup10TCATGCCACC small insertion frequently observed in PCG. This variation results in a frame shift. We present a novel mutation in PITX2 and report for the first time CYP1B1 mutations as causes of the complex symptoms of Riegers anomaly.

Gene expression analysis in patients with defects in RNA splicing factors causing autosomal dominant retinitis pigmentosa. *C. Rivolta*¹, *R.V. Jensen*^{2,3}, *E.L. Berson*¹, *T.P. Dryja*¹. 1) Department of Ophthalmology, Harvard Medical School, Mass. Eye and Ear Infirmary, Boston, MA; 2) Department of Neurology, Harvard Medical School, Brigham and Women's Hospital, Boston, MA; 3) Department of Physics, Wesleyan University, Middletown, CT.

Dominant mutations in the genes RP11 (PRPF31), RP13 (PRPC8), and RP18 (HPRP3), all encoding RNA splicing factors, cause retinitis pigmentosa (RP), a form of retinal degeneration leading to blindness. RP11 mutations are incompletely penetrant. Through an analysis of patients with mutations in these genes, we investigated the molecular link between an ubiquitous biological process (RNA splicing) and a tissue-specific phenotype (RP).

We harvested RNA from cultured lymphoblast cell lines from individuals with mutations in RP11 (N=10, 3 unaffected), RP13 (4), RP18 (3), and controls (7). We used real-time PCR, qualitative RT-PCR, microarray hybridization (Affymetrix and Codelink), and in silico tests to assess variations in gene expression and differences in gene splicing.

Expression of spliced genes, normalized to single-exon histone mRNA transcripts, indicated no generalized impairment of the RNA splicing in all patients vs. all controls (difference between the medians <0.1%). Unsupervised cluster analyses showed no major branching. However, we did find that the expression of the wt RP11 allele was higher in heterozygous carriers of mutations than individual wt alleles in controls (~80% higher in 3 unaffected individuals with 2 different mutations, versus only ~40% higher in 7 affected individuals with 6 different mutations).

These results suggest that death of photoreceptors does not occur because of an indiscriminate reduction of global RNA splicing. In addition, the analysis of RP11 transcripts indicates that penetrance in patients with RP11 mutations is associated with variation in the expression level of the wild-type RP11 allele itself. This phenomenon likely compensates the currently obscure functional deficiency caused by the mutant allele.

Cosegregation analysis of mutant ABCA4 alleles in families with both Stargardt disease (STGD) and age-related macular degeneration (AMD). JM. Rozet¹, D. Ducroq¹, S. Gerber¹, JL. Dufier², A. Munnich¹, J. Kaplan¹. 1) INSERM U393, Hopital des Enfants Malades, Paris, CDX 15, France; 2) Service de Genetique, Hopital des Enfants Malades, Paris, France.

The study presented aimed to evaluate whether AMD-affected relatives of Stargardt patients are more likely to be carriers of mutant STGD alleles than predicted based only on their position in the pedigree. Sixteen unrelated families segregating both Stargardt disease and AMD were considered. In one STGD patient of each family, the ABCA4 gene was screened for the most frequent mutations in France. A segregation analysis was subsequently performed for each mutant allele in AMD-affected relatives. The observed proportion of AMD-affected relatives ABCA4 mutation carriers was compared to the expected proportion based on chance alone. So far, at least one of the most frequent ABCA4 mutations in France was found in 8/16 families. Of the ten AMD-affected relatives belonging to these eight families, 9/10 were found to be carriers of the disease allele, therefore $P_{\text{observed}} = 0.90$ while based on the genealogy only, the $P_{\text{theoretical}}$ was equal to 0.431. These proportions were compared using the Chi square test. The difference between P_{observed} and $P_{\text{theoretical}}$ is statistically significant ($\chi^2 = 9.02$, $p < 0.01$). The first results of this study indicate that ABCA4 mutation carriers are predisposed to develop AMD more frequently than the general population. The whole ABCA4 coding sequence screening is ongoing in all families using both DHPLC and direct sequencing.

A novel Filamin A D203Y point mutation in a female patient with Otopalatodigital type 1 syndrome and extremely skewed X chromosome inactivation. *A. Bravo¹, J.C. Zenteno-Ruiz¹, S. Kofman-Alfaro¹, E.N. Pompa-Mera², C. Gonzalez-Bonilla².* 1) Servicio de Genética, Hospital General de México, Facultad de Medicina, UNAM; 2) UIM en Inmunología e Infectología, CMN La Raza, IMSS.

Otopalatodigital syndrome type I (OPD I) is an X-linked semidominant disease, characterized by mild mental retardation, hearing loss, cleft palate, facial anomalies and a generalized skeletal dysplasia. Other three X-linked entities displaying features that overlap clinically with OPDI have been described and named OPD II, Melnick-Needles Syndrome and Frontometaphyseal Dysplasia. Based on this clinical overlap, several authors have proposed that the four entities are allelic disorders. The molecular basis of these four entities have shown mutations in the gene encoding the cytoskeletal protein filamin A (FLNA). Herein, we present the clinical and molecular data of a sporadic female patient clinically diagnosed with an OPD I syndrome. She consulted at 26 years old and clinical examination revealed short stature, facial features included prominent supraorbital ridges, hypertelorism, downslanting palpebral fissures, depressed nasal base, median cleft soft palate and bilateral conductive hearing loss. Bilateral elbow deformity, brachydactyly with broad thumbs, and short dysplastic nails were observed in upper extremities. In lower limbs she presented bilateral femoral bowing, short and broad great toes, gap between the second and third and between the third and fourth toes, as well as nail dystrophy. X rays demonstrated thickness of the frontal bone, bowing of long tubular bones, and short and broad distal phalanges in thumbs and great toes. A GTG-banded chromosome analysis from blood lymphocytes revealed a normal 46,XX karyotype. DNA analysis demonstrated that she carried a novel FLNA heterozygous point mutation (D203Y) that affects the actin binding domain of the protein. X-chromosome inactivation analyses demonstrated an extremely skewed pattern of inactivation. Our findings confirm an ample molecular spectrum of human malformative syndromes caused by FLNA mutations and contribute to delineate an incipient phenotype-genotype correlation in this group of X-linked disorders.

Mutation analysis in a cohort of 78 cases of Oral-facial-digital syndrome: an international collaborative effort. *B. Franco, V. Novelli, G. Giorgio, A. Barra, The international OFD collaborative research group. TIGEM, Naples, Italy.*

Oral-facial-digital syndromes (OFDs) are a heterogeneous group of developmental disorders of which at least nine different forms have been described to date. The different forms are very similar from a clinical point of view and it is difficult to ascertain the exact subtype. The only well-defined form is OFD type I (OFDI), which can be recognized for the distinctive X-linked dominant male-lethal pattern of inheritance and for the presence of a polycystic kidney. The identification of the gene responsible for OFDI has provided an important tool for genetic counseling and a molecular test is now available to patients and clinicians for OFDI diagnosis and prenatal testing. To determine the extent of the involvement of the OFD1 gene in OFDs, and to better define both the clinical spectrum of Oral-facial-digital type I syndrome and the actual number of different OFDs, we collected through an international collaborative effort a cohort of 78 patients which includes typical OFD1 and cases with more general signs of oral-facial-digital syndromes. Mutation analysis for the OFD1 gene was performed in this collection on the canonical 23 coding exons and on the alternative spliced exons by DHPLC followed by direct sequencing. The analysis has been so far completed for 65 out of 78 patients. A total of 50 mutations have been identified, these include 7 missense, 9 nonsense, 30 frameshifts and 4 splicing mutations. Mutations occur throughout the length of the transcript up to exon 16, although the majority (62%) were located in exons 3(20%), exon 8 (18%), exon 9 (10%), exon 16 (8%), exon 13 (6%). So far no clear genotype/phenotype correlation could be established. To be noted that according to our preliminary results the incidence of polycystic kidney is underestimated in this pathology. In fact, 17 out of the 49 patients in which a mutation in the OFD1 gene has been identified and for which clinical information are available have cystic kidneys, thus indicating an incidence of polycystic kidney in this condition of at least 34%.

***RPGR*, a gene mutated in patients with a complex phenotype associating X-linked primary ciliary dyskinesia and retinitis pigmentosa.** A. Moore¹, E. Escudier², G. Roger³, S. Marlin³, A. Tamalet³, M. Geremek⁴, A. Coste², M. Witt⁴, B. Duriez¹, S. Amselem¹. 1) INSERM U468, Creteil, France; 2) INSERM U492, Creteil, France; 3) Hopital Trousseau, Paris, France; 4) Institute of Human Genetics, Poznań, Poland.

Primary Ciliary Dyskinesia (PCD) is a rare disease classically transmitted as an autosomal recessive trait and characterized by recurrent airway infections due to abnormal ciliary structure and function, usually involving dynein arms. To date, only two autosomal genes, *DNAI1* and *DNAH5* encoding axonemal dynein chains have been shown to cause PCD with a total lack of outer dynein arms. We studied one non-consanguineous family composed of four children, with two boys with PCD. Patients cilia displayed an abnormal motion related to partial ciliary defects. Electroretinograms revealed that the two boys and their mother were suffering from retinitis pigmentosa (RP). These data are, therefore, compatible with an X-linked recessive transmission of PCD and RP diseases, in keeping with recent data (Krawczynski and Witt, *Pediatr. Pulmonol.* 2004;38(1):88-9). As photoreceptor and respiratory cilia share common structures, we postulated that some genes responsible for RP could also be involved in PCD. We found a perfect segregation of the disease phenotype with RP3 locus (Xp21.1). The analysis of the *RPGR* gene located at this locus showed a deletion involving the last 48bp of exon 6 and the adjacent splice site in the two boys and their mother. This deletion leads to activation of a cryptic donor splice site located within exon 6, predicting a severely truncated protein. Overall, these data provide the first clear-cut demonstration of an X-linked transmission of PCD. This unusual mode of inheritance of PCD is described in patients with particular features (i.e. partial dynein arm defects and association with RP), pointing out the importance of *RPGR* in the proper development of both axonemal structures and connecting cilia of photoreceptors. Ocular signs characteristic of RP should, therefore, be looked for in patients with PCD and vice versa.

Towards dissection of the molecular basis of Microphthalmia with linear skin lesion (MLS) syndrome. *M. Morleo*¹, *T. Pramparo*², *A. David*³, *A. Raas-Rothschild*⁴, *M. Vekemans*⁵, *G. Zaidman*⁶, *T. Ogata*⁷, *O. Zuffardi*², *A. Ballabio*¹, *B. Franco*¹. 1) TIGEM, Naples, Italy; 2) Biologia Generale e Genetica Medica, Pavia, Italy; 3) Service de Genetique medicale, Nantes, France; 4) Department of Human Genetics Hadassah University, Jerusalem, Israel; 5) Hopital Trousseau, Paris, France; 6) Department of Ophthalmology, New York Medical College and Westchester Medical Center, Valhalla, NY, USA; 7) Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan.

Microphthalmia with linear skin defects (MLS) is an X-linked dominant male lethal neurodevelopmental disorder characterized by microphthalmia and linear erythematous skin lesions on the face and neck. Additional findings include sclerocornea, corneal opacities, brain abnormalities and mental retardation. This condition described in the early 90 has been associated with monosomy of the Xp22.3 region. The minimal critical region defined by deletion mapping, is estimated to span 600kb and is entirely sequenced. We have now collected and characterized by FISH analysis with available genomic clones eight newly identified MLS patients bearing cytogenetic abnormalities of the Xp22 region. Characterization of these breakpoints confirmed the critical region previously defined. Moreover we collected four patients displaying signs of MLS with a normal karyotype. FISH analysis with clones spanning the minimal critical interval (BAC/PAC/cosmids) excluded monosomy of the Xp22 region. Mutation analysis in these patients is currently ongoing to evaluate the possible involvement of genes previously isolated from the critical region (MID1, HCCS and ARHGAP6) as well as of candidate genes identified by bioinformatics means. To investigate the possibility of altered expression levels of genes localized within the MLS minimal critical region or in the surrounding area we are also performing a quantitative analysis of gene expression levels by Real-Time RT-PCR on total RNAs from MLS patients with normal karyotype. Genes to be analysed include MID1, HCCS, ARHGAP6, MSL3L1, PRPS2, TLR7, TLR8, EGFL6, AK097032, RAB9A.

Male patients with Rett syndrome-like phenotype do not present mutations in the *MECP2*, *NLGN3* and *NLGN4* genes. M. Santos^{1,2}, T. Temudo^{2,3}, C. Barbot⁴, A. Medeira⁵, H. Cabral⁶, P. Cabral⁷, I. Carrilho⁴, I. Gaspar⁷, N. Lobo-Antunes⁵, A. Moreira⁸, J. Sequeiros^{2,9}, P. Maciel^{1,2}. 1) ICVS/School Health Sciences, Univ Minho, Braga, Portugal; 2) ICBAS, Univ Porto; 3) H S António, Porto; 4) H M Pia, Porto; 5) H Sta Maria, Lisboa; 6) C H Funchal, Funchal; 7) H Egas Moniz, Lisboa; 8) H D Estefânia, Lisboa; 9) UnIGENE/IBMC, Univ Porto.

Numerous genes in the X chromosome have been reported as having a role in autistic spectrum disorders, which can be wide and include disorders such as autism with or without mental retardation, Asperger syndrome, Rett syndrome (RS), X-linked mental retardation among others. Among these are two neuroligin genes, *NLGN3* and *NLGN4* whose protein products are present in the postsynaptic compartment, interact with neuroligins and are thought to be essential in the formation of specific synapses. Mutations have also been identified in the *MECP2* gene, in patients with classical RS and Rett-like phenotypes. The MeCP2 protein binds selectively to methylated DNA and interacts with Sin3a and HDAC to repress transcription of genes, yet to be identified. We studied a group of 12 male patients (ages 6 to 19 years) whose biological samples were received in our laboratory to be tested for mutations in the *MECP2* gene. Clinically, these patients presented a RS-like phenotype with variable symptoms that included perturbations of the autistic spectrum, microcephaly, mental retardation, manual stereotypies, and neonatal epilepsy (West syndrome). We analysed this group of male patients for the complete coding region of the *MECP2* gene and for the recently reported mutations in the *NLGN3* (R451C) and *NLGN4* (1186insT and 1253delAG) genes. We did not find any pathogenic mutation in *MECP2*, *NLGN3* or *NLGN4* genes. A silent polymorphism (T299T), already described, was found in the *MECP2* gene of one patient. Another patient presented a silent mutation in the *NLGN4* gene (R417R) reported here for the first time which we are presently testing in a Portuguese control population. The genetic basis of the clinical diagnosis of these male patients was therefore not possible to identify and these results suggests that additional genes may be involved in this kind of disorders.

Genotype-phenotype correlations in Portuguese patients with Rett syndrome. T. Temudo^{1,2}, M. Santos^{2,3}, K. Dias⁴, C. Barbot⁵, P. Cabral⁶, A. Moreira⁴, I. Carrilho⁵, N. Lobo-Antunes⁷, G. Oliveira⁸, J. Sequeiros^{9,2}, P. Maciel^{2,3}. 1) Neuropediatrics, HGSA, Porto, Portugal; 2) ICBAS, Univ Porto; 3) ICVS/Health Sciences School, Univ Minho; 4) H D Estefnia, Lisboa; 5) H M Pia, Porto; 6) H Egas Moniz, Lisboa; 7) H Sta Maria, Lisboa; 8) C H Coimbra, Coimbra; 9) UnIGENe/IBMC, Univ Porto.

Rett syndrome (RS) is a neurodevelopmental disorder caused by mutations in the X-linked gene *MECP2*, which is characterized by an initially normal development of the child, followed by loss of acquisitions and growth impairment, which evolve into a clinical presentation including mental retardation, autistic behaviours and a progressive movement disorder. We have initiated the study of the genetic epidemiology of this disorder in Portugal and have analysed 61 girls, all of which were observed by the same neuropediatrician, and recorded on video. This series included 31 girls with the clinical diagnosis of classic Rett syndrome, and the remaining with variant forms of the disease. We have sequenced the complete coding region of the *MECP2* gene and identified *de novo* mutations in 37 (61%) of the patients; 22 (60%) were truncating, and 15 (40%) were missense mutations. We have tried to correlate the clinical severity of the disease with the type of mutation identified, using the Pineda score, however no association was detectable between the total score and mutation type, even when patients with the same time of disease evolution were considered. We could, however, detect an association between the type of mutation and some clinical hallmarks of RS, such as the presence of microcephaly (significantly more frequent among carriers of truncating mutations, Fisher exact test 0.0043) and the ability to walk (significantly more frequent among carriers of missense mutations, Fisher exact test 0.049).

Congenital Insensitivity to Pain with Anhidrosis (CIPA): A novel mutation among Marocon Jews. *T.C. Falik-Zaccai*^{1, 2}, *M. Khayat*¹, *M. Veiller*¹, *N. Kfir*¹, *M. Deinzer*³, *C. Aslanidis*³, *G. Schmitz*³. 1) Medical Library, Western Galilee Hosp, Nahariya, Israel; 2) Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel; 3) Institut Fur Humangenetik, Universitätsklinikum der RWTH Aachen, Germany;.

Congenital Insensitivity to Pain with Anhidrosis (CIPA) is a rare autosomal recessive genetic disorder characterized by congenital insensitivity to pain, temperature sensation defect, mental retardation, self-mutilating behavior and inability to sweat. The anomalous pain and temperature sensation and anhidrosis in CIPA are due to the absence of afferent neurons activated by tissue-damaging stimuli and a loss of innervation of eccrine sweat glands, respectively. The NTRK1 gene located on chromosome 1 encodes a receptor tyrosine kinase which is autophosphorylated in response to nerve growth factor, thus, activating various pathways of intracellular signal transduction. Fourty mutations have been reported so far in the NTRK1 gene as causing CIPA. We report a novel two-nucleotide deletion mutation within the coding region of NTRK1 that result in frame shift very early in the protein and generates a premature stop codon. The mutation was detected at a homozygous state in two siblings affected with CIPA who are of Marocon Jewish origin. The non-consanguineous parents and a healthy brother were found to carry the same mutation. The CIPA causative mutation was not detected in 120 control DNA samples from Marocon Jews. The family requested prenatal diagnosis as soon as the causative mutation was identified. The fetus was found to be a heterozygous carrier of the causative mutation. This is the first report of a causative mutation for CIPA within unrelated Marocon Jews and the second time PND is documented. The prevalence of this mutation among this homogeneous ethnic group seems to be low. These results will enable to detect couples at risk within the extended family, and to provide accurate genetic counseling and prenatal diagnosis for interested couples. Identification of new mutations and the study of their pathologic impact in CIPA can serve as a useful model to determine mechanisms of development and maintenance of NGF-dependent neurons in autonomic, sensory and central nervous system in humans.

Spectrum of mutations in the CLCN1 gene in Finnish Myotonia congenita patients. *K. Kantojarvi, M-L. Vaisanen, J. Ignatius.* Dept of Clinical Genetics, Oulu University Hospital, Oulu, Finland.

Myotonia congenita (MC) is an inherited muscle disease resulting from hyperexcitability of the skeletal muscle membrane. Both autosomal dominant (Thomsen disease) and more frequent recessive (Becker disease) are caused by mutations in the skeletal muscle chloride channel gene (CLCN1). CLCN1 is mapped to chromosome 7q35 and consists of 23 exons. So far over 60 mutations has been identified, which distribute over the entire gene. No mutational hotspot regions or specific mutations with a dominant or recessive inheritance have been detected.

In northern Finnish and northern Scandinavian MC-families three common CLCN1 mutations are found (F413C, A531V, R894X). During the years 1999-2004 we have tested these three mutations in 92 patients suspected of having MC; 24 originating from North Finland and 68 sent from other parts of the country. Homozygosity or compound heterozygosity has been detected in 8/24 (33%) northern Finnish cases and in 17/68 (25%) cases coming elsewhere. In addition, several patients with mutation in only one allele has been found, which although confirming the diagnosis does not give information of the inheritance pattern, especially if family data is lacking. Compound heterozygosity of the other unknown mutation contributing to the phenotype could be the likely explanation in these cases.

In order to find out the possible other mutations, we have started heteroduplex scanning by conformation-sensitive gel electrophoresis (CSGE) of all CLCN1 exons with flanking sequences followed by direct sequencing. The material consists of 22 clinically symptomatic MC patients (3 from North Finland and 19 from other parts of Finland) with one of the common mutations found in routine mutation analysis. In addition, 44 cases with no mutations found previously are included. On the cases analysed, variant fragments has been found by CSGE in several exons, which are currently being sequenced. Several new as well as known sequence variants and/or mutations (missense, splice site, frame shift) has been found and their further characterization is in progress.

Mutation detection of CFTR gene using ARMS and SSCP methods in Iran. *M. Mojarrad¹, N. Yazdanpanahi¹, P. Derakhshandeh-Peykar^{1,2}, M. Rafiee³, R. Mirfakhraie¹, M. Jalali¹, M. Rahmani¹, D. Farhud¹, M. Hashemzadeh¹, F. Mirzajani¹, F. Mirzajani⁴.* 1) school of Public Health, Tehran, Iran; 2) Genetic Clinic, Valie-Asr Sq. 16, Keshavarz blvd., Tehran, Iran; 3) Tabriz Pediatrics Hospital, Tabriz-Iran, Tehran, Iran; 4) National Genetic Engineering and Biotechnology Center, Tehran, Iran.

Cystic fibrosis (CF) is the most common severe lethal autosomal recessive disorder in whites that is caused by mutations occurring in the CFTR gene on chromosome 7q31. The carrier frequency among Caucasians is approximately 1 in 25, with an incidence of approximately 1 in 2500 live birth. CF patients have two defective alleles and may either be heterozygous for different mutations, or homozygous for one of the mutations. Since the identification of the gene responsible for CF, more than 1300 mutations was described in CFTR gene of patients affected by cystic fibrosis, but the prevalence of the CF shows a geographical and ethnical variations in the world. The Delta F508 mutation in CFTR gene accounts for over 70 % all mutant CFTR alleles in the Europeans to 20 % in the Asians. First the DNA samples of 92 CF chromosomes have been tested for eight common mutations: F508, W1282X, 621+1G>T, 1717-1G>A, G542X, G551D, N1303K and R560T by using the ARMS method. Results from this study revealed the following frequencies: F508: 19.5%, G542X: 10%, W1282X: 6.5%, N1303K: 1.1%, 621+1G>T: 0%, 1717-1G>A: 0%, G551D: 0%, R560T 0%. Then by using SSCP method for samples which we didnt find any mutation by ARMS system, we scanned exons 4, 7, 10, 11 and intron 11 of CFTR gene. By using sequencing we find two mutations in exon 4 (R117H: one chromosome, and A120T: one chromosome), one mutation in exon 7 (R347H: one chromosome) and a novel mutation in intron 11 (1811+34 A>G: one chromosome).

Friedreich ataxia mouse models demonstrate an absence of oxidative stress concomitant to a reduction of SOD2 expression. *H. Puccio, H. Seznec, D. Simon, L. Reutenauer, M. Koenig.* Molecular Pathology, IGBMC, Illkirch, Strasbourg, France.

Friedreich ataxia (FRDA), a progressive neurodegenerative disorder associated with cardiomyopathy is caused by severely reduced frataxin, a mitochondrial protein involved in Fe-S cluster assembly. Iron-mediated oxidative stress and impairment of the stress defense proteins superoxide dismutases (SODs) have been suggested to be implicated in the pathology of FRDA. This has led to the development of pharmacological therapy using antioxidants or iron chelators. Indeed, there are evidences that idebenone, a coenzyme Q10 analog, can improve cardiac function in FRDA patients. We have generated conditional mouse models that reproduce important progressive pathological and biochemical features (cardiac and neuronal) of the human disease. We reported that idebenone has a significant effect on the cardiac function and the life-span of the murine model, with a significant reduction of oxidative stress indexes concomitant to mitochondrial iron accumulation suggesting that the iron is non-toxic. Oxidative stress markers in neuronal tissues are not change despite the presence of a pathological process. Furthermore, tetrakis-(4-benzoic acid) porphyrin (MnTBAP), a synthetic SOD mimetic, had no significant effect on the survival of the cardiac model at a dose sufficient to rescue a SOD2 null mouse. We found that there was a significant reduction in SOD2 expression and activity in tissues deleted for frataxin, suggesting that the reduced SOD2 expression by a yet unknown mechanism is probably responsible for the lack of SOD2 induction in FRDA. Taken together, these results clearly demonstrate that iron-mediated oxidative stress is not a key pathological component of the disease, and that efforts in finding new therapeutics molecules for FRDA should therefore not be limited to antioxidant and iron chelators. Furthermore, our results suggests that frataxin function is central to the cell and that it is not only necessary for the proper maintenance of the respiratory chain through Fe-S cluster assembly, but also necessary for the proper expression of the stress defense protein SOD2.

Neonatal screening of inherited non-syndromic congenital deafness: results of a pilot program in South Brazil. E.

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Congenital deafness is estimated to occur in 1 of each 1,000 births. In about 70 % of the cases, deafness appears as an isolated sign and is called non-syndromic (NSD). When not early identified and treated, NSD leads to language development problems, usually severe. Mutations in several genes have been implicated on the etiology of NSD. However, a single mutation (35delG) on DFNB1, the gene that codifies Connexin 26, seems to be very frequent and could be an important cause of NSD. Our group adapted a method to extract DNA from dried-blood filter paper samples (already collected for newborn screening of metabolic diseases). We used an in-house method (based on methanol extraction) and set up a user-friendly and low-cost molecular-based technique to detect 35delG mutation, suitable for mass screening. A pilot program was carried out on DBFP samples obtained from 100 newborns. The 35delG mutation was found in 4/200 alleles (2%), indicating that the frequency of NSD due to 35delG mutation on the DFNB1 gene could be as high as 1/10,000, even higher than PKU on our region, and should be considered for introduction on neonatal screening programs for congenital diseases.

A new genetic screening test for hereditary hearing loss. *N. Navot, D. Cohen, M. Mandelman, A. Yakir, A. Eran.*
Pronto Diagnostics Ltd, Rehovot, Israel.

Profound hearing loss is the most common congenital disability in the United States; it occurs in three out of every 1000 births. 12,000 infants are born with permanent hearing loss annually, with more than 60% attributed to genetic factors. Recognizing the importance of early detection of childhood hearing loss, 38 states have passed legislative mandates requiring newborn hearing screening; most include the American College of Medical Genetics recommendation that children with confirmed hearing loss be referred for genetic testing and counseling. Nonsyndromic, recessively inherited deafness is the predominant form of inherited deafness. It is caused by mutations in the Connexin 26 (GJB2) and Connexin 30 (GJB6) genes, located at the DFNB1 locus. Recent advances in molecular genetics resulted in the addition of DFNB1 genotyping to classical Ashkenazi Jewish prenatal diagnosis panels. We have developed a large-scale screening assay for the simultaneous detection of DFNB1's three most common mutations, based on the Pronto technology. Pronto Connexin is a multiplex mutation detection method based on a single nucleotide primer extension reaction, followed by ELISA in a kit format. Each sample is tested in four wells of a 96-well microtiter plate for the 35delG and 167delT mutations in the GJB2 gene and the (GJB6-D13S1830) mutation in the GJB6 gene. This efficient screening format presents the complete genotype of the common 35delG mutation and a carrier status of the latter less frequent mutations, whose complete genotype can be detected using the Pronto Connexin Verification Strip. Each detection well contains a 5' labeled primer complementary to the upstream sequence of the mutation site, and a single biotinylated nucleotide. The primer is extended depending on the tested individual's genotype, thus becomes labeled with biotin. Reaction products are transferred to a streptavidin-coated plate and treated according to a standard ELISA protocol. Results are determined either visually or colorimetrically (O.D. 450 nm). Pronto Connexin was designed to meet the emerging needs for rapid large-scale newborn screening tools. This assay completes a 33-mutation panel yielding a uniform diagnostic tool for Ashkenazi Jewish carrier screening.

Pre-implantation Genetic Diagnosis (PGD) for Genetic Disorders in Saudi Arabia. *A.I. Al-Aqeel^{1, 2}, S. Coskun², K.A. Jaroudi², P.T. Ozand², A. Hellani².* 1) Dept Pediatrics, Riyadh Military Hosp, Riyadh, Saudi Arabia; 2) King Faisal Specialist Hospital & Research Centre.

Saudi Arabian culture is highly consanguineous, with the first cousin marriages accounting for 60-70% of all marriages. Given the difficulties in management of genetic disorders, preventive measures for the suffering families from autosomal recessive disorders by doing pre-implantation genetic diagnosis is undertaken. The first of these disorders is Sanjad-Sakati Syndrome (SSS) OMIM# 24140, which is characterized by congenital hypoparathyroidism, growth and mental retardation with a unique 12bp deletion (nucleotide 156-166) in Tubulin Cofactor E (TBCE) gene. The second is Niemann Pick Disease type B (NPD-B) OMIM# 257200,(acid sphingomyelinase (ASM) deficiency) with more than 70 mutations have been reported in (SMPD1) gene, which presents with severe phenotype in Saudi Arabia. Four unique mutations are found in our Saudi families. A family with (W533R) mutation in the (SPMDI) gene suffering from a severe phenotype underwent PGD. The third disorder is Morquios disease (MPSIV) OMIM # 253000, with severe classic phenotype with N-acetyl galactosamine-6-sulfatase deficiency (MPSIV-A). More than 20 different mutations in (GALNS) gene have been reported in (MPSIV-A). A family with three affected siblings with severe classic (MPSIV-A) with detected W195C mutation in the (GALNS) gene underwent PGD. In all these three families PGD was undertaken using fluorescent PCR(F-PCR) and/or nested PCR with sequencing on a single cell. A singleton pregnancy ensued after transfer of one heterozygous and one normal embryo and prenatal diagnosis by CVS confirmed a normal pregnancy. This is the first report of successful PGD in different genetic disorders in Saudi Arabia.

Karyotyping, immunophenotyping, and apoptosis analyses on human hematopoietic precursor cells derived from umbilical cord blood following long-term ex vivo expansion. *Z. Chen^{1,2,3}, H. Tian¹, S. Huang², F. Gong¹, L. Tian⁴.*

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Umbilical cord blood (UCB) has been considered an attractive alternative to bone marrow or mobilized peripheral blood for hematopoietic precursor cells engraftment. However, the number of hematopoietic stem cells (HSC) in UCB is limited. Ex vivo expansion of UCB-HSC and the stability of their biological and genetic characteristics under incubation are essential for successful UCB-HSC transplantation. However, there has been lack of extensive systemic investigations on the biological and genetic nature of UCB-derived CD34⁺/CD38⁻ stem cells following long-term ex vivo expansion. By flow cytometry CD34⁺/CD38⁻ HSC were collected from UCB of 10 healthy women at the time of delivery and cultivated in stem cell culture media supplemented with cell growth stimulating factors (IL-3,IL-6,GM-CSF,EPO,IGF-1, and SCF) for a long-period of time. In our experiments, the UCB-derived CD34⁺/CD38⁻ stem cells were able to divide and proliferate in vitro for at least six months. They did not show significantly increased apoptosis following ex vivo expansion for 20 and 32 days, respectively in two cases tested and remained the same cell surface marker expression pattern (i.e., CD34⁺ and CD38⁻) in majority of the cells of two cases tested following 20 and 37 days of incubation, respectively. In another two cases tested, chromosome analysis showed no evidence of apparent numerical and structural abnormalities on the CD34⁺/CD38⁻ stem cells obtained after 20 and 43 days in culture, respectively. Our findings indicated that UCB-derived CD34⁺/CD38⁻ stem cells are able to maintain their basic biological and genetic characteristics after proliferating in vitro over a long-period of time. UCB-derived HSC following ex vivo expansion can serve as a reliable resource for HSC transplantation.

Labor increases maternal DNA contamination in cord blood. *K. Miura, H. Masuzaki, S. Miura, K. Hiraki, S. Yoshimura, D. Nakayama, T. Ishimaru.* Dept OB/GYN, Nagasaki Univ School of Med, Nagasaki, Nagasaki, Japan.

Objective: Although maternal cells and deoxyribonucleic acids (DNAs) were reported to be present in umbilical cord blood (UCB), which factors affect on this umbilical cord blood contamination by maternal genetic materials remains unknown. In this study, we aimed to know whether labor itself influences the frequency of maternal DNA contamination in UCB. Material and Method: Ninety-seven pregnant women with normal pregnancy were divided into 19 Group-A women with C/S performed after the beginning of labor (labor with C/S), 44 Group-B women with C/S before labor (non-labor with C/S), and 34 Group-C women with normal vaginal delivery (V/D) (labor with V/D). A set of 9 highly polymorphic tetra-repeats markers in Japanese population (D21S11, D18S535, D11S2365, D8S1132, D7S1830, D4S1645, D3S1359, D3S2465 and FGA) was chosen for detection of maternal DNA. We compared the detection rate of maternal DNA in cord plasma samples between three groups. Difference in categorical variables was compared by chi-squared test, and difference between continuous variables was compared with Mann-Whitney U test. A P value .05 was considered significant. Results: When maternal DNA had 2 alleles (the mother, heterozygous) and one of them was not shared by UCB (fetal) cellular DNA, we defined this allele as informative. The presence of maternal DNA in UCB was judged when an informative allele is found for at least 2 different loci. We compared detection rates of maternal DNA in UCB plasma between the 3 groups of women. Consequently, the detection rate (16/19) in Group A was significantly higher than that (20/44) in Group B (p 0.005). Likewise, the rate (31/34) in Group C was statistically higher than that (20/44) in Group B (p 0.0001). However, there was no significant difference in the rate between Groups A and C (p 0.5). Conclusion: It became clear that labor affects on the frequency of the cord blood contamination by maternal DNA. The results also imply clinical importance of the use of cord blood at bone marrow transplantation, and may give an insight into the mechanism of feto-maternal circulation and vertical transmission of infectious agents.

Human chondrocytic lines provide a model for achondroplasia and thanatophoric dysplasia. *C. Benoist-Lasselien, J. Bonaventure, L. Gibbs, S. Heuertz, A. Munnich, L. Legeai-Mallet.* INSERM-U393, Hopital Necker, Paris, France.

Achondroplasia (ACH) and thanatophoric dysplasia (TD) are human skeletal disorders of increasing severity accounted for by mutations in the fibroblast growth factor receptor 3 (FGFR3) gene. We generated six immortalized human chondrocyte lines that express a constitutively heterozygous mutant of FGFR3. Mutation analyses showed that the chondrocytic lines carried respectively the G380R mutation (ACH phenotype), the S249C, R248C, G370C, Y373C mutations (TDI phenotype) and the K650E mutation (TDII phenotype). Chondrocytes were isolated from human fetal growth cartilage and immortalized by transfection of the SV40 large T antigen gene. The cell lines were characterized and analyzed for factors controlling chondrocyte differentiation. Cell lines, were subcloned according to the following parameters : cell morphology, mRNA and protein levels of extracellular matrix molecules to confirm a cartilage-specific and stable phenotype. We selected cell lines associated with an expression of extensive extracellular matrix components including proteoglycans (aggrecan, biglycan, decorin), collagens type II and type IX, MMP9 and signaling molecules (Ihh, Pthrp, FGFR3). Here we show the constitutive phosphorylation of FGFR3 and the activation of the STAT pathway in these immortalized cells. The cell lines provide a good model for ACH, TDI and TDII phenotypes, in addition we show for the first time the excessive activation of signaling cascades mediated by the FGFR3 mutants in human chondrocytic cell lines. Availability of this model will permit rational strategies for targeting the FGFR3 signaling pathways and to address new strategies in the treatment of achondroplasia. This work was supported by the European Skeletal Dysplasia Network (ESDN), grant CE-QLG1-CT-2001-02188.

Radiographic diagnosis of osteogenesis imperfecta type II following atypical ultrasound findings. *S.M. Carter, D. Cordero, S. Klugman, J.F. Powers, S.J. Gross.* Reproductive Genetics, Montefiore Medical Ctr, Bronx, NY.

A 19-year-old African primigravida had appeared for a routine fetal anatomy scan at 18 weeks' gestation. Initial ultrasound findings revealed a single live fetus with severe micromelia of the upper and lower limbs along with abnormally shaped ribs. A small chest cavity resulted in cardiac displacement. After the patient was counseled about the grim prognosis for the pregnancy, she requested a pregnancy termination. An ultrasound by an experienced fetal dysmorphologist confirmed the initial anomalies and demonstrated decreased bone mineralization. The ribs did not appear beaded and bone fractures were not observed. The differential diagnoses included osteogenesis imperfecta, achondrogenesis, hypophosphatasia, and thanatophoric dysplasia.

After pregnancy termination, the fetus was evaluated by the International Skeletal Dysplasia Registry (Cedars-Sinai Medical Center, Los Angeles, CA). Posttermination radiographic findings were consistent with osteogenesis imperfecta (OI) type II, a type 1 collagenopathy. The fetus had osteopenia, crumpled femurs, and other long bones with beaded ribs. The triad of bone shortening, hypomineralization, and numerous fractures are pathognomonic for OI type II. However, not all three features need be present simultaneously as fractures may not develop until later. Additionally, the fractures may have arisen iatrogenically from the dilation and evacuation procedure performed at 19 weeks. We discuss the natural history, molecular biology, and variable ultrasonographic findings of OI type II as well the recurrence risk and prenatal diagnosis modalities for future pregnancies.

Cystic Hygroma and Arthrogryposis Multiplex Congenita: An additional case. *C. Cheng¹, V. Shah¹, P. Shah¹, R. Windrim², M. Bedford⁴, D. Chitayat^{2,3}.* 1) Department of Paediatrics; 2) Department of Obstetrics and Gynecology; 3) The Prenatal Diagnosis and Medical Genetics, Mount Sinai Hospital; 4) Department of Medical Genetics, North York General Hospital, Toronto, ON, Canada.

Arthrogryposis multiplex congenita (AMC) is a term used to describe multiple joint contractures detected at or before birth. The etiology is heterogeneous and may be a presenting feature of various conditions. Cystic hygroma (CH) is a heterogeneous condition caused by abnormalities in the lymphatic system. Scott et al reported the combination of both findings once.⁽¹⁾ We report a case of this combination of findings. The infant was born at 38.5 weeks GA to a 34-year-old G4P3L3 woman of Italian descent. The couple's family histories were noncontributory. Fetal US at 18.5 weeks gestation showed CH with clenched fists. On an US repeated a week later, the fetal fingers remained clenched with no overlapping and both hands were held in extreme flexion position. Rest of the scan was normal. Amniocentesis showed a normal karyotype (46 XX) and negative DNA analysis for PTPN11 ruling out Noonan syndrome. Fetal ECHO was normal and MRI showed normal brain maturation and structure and confirmed the presence of CH. The infant was born by Cesarean section for breech position with Apgar scores of 6 and 9 at 1 and 5 minutes. On examination the infant was hypotonic, had myopathic face, droopy eyelids, retrognathia, low nuchal hair line, webbed neck, flexion contractures of both wrist joints, camptodactyly of the 2-5th fingers, partial syndactyly of the 2nd and 3rd toes, hammer great toe, hyperflexed feet and limitations in extension of both knees. Rest of the examination was normal. To date there has been one published case report of non-lethal AMC with a CH diagnosed at 13 weeks gestation.⁽¹⁾ The two female siblings had a similar presentation and the authors hypothesized an autosomal recessive condition. Our case supports the assumption that the combination is most probably a single gene disorder with a triad of CH, AMC, and hypokinesia. Reference: 1. Scott H, Hunter A, Bedard B. Non-lethal arthrogryposis multiplex congenital presenting with cystic hygroma at 13 weeks gestational age. *Prenat Diagn* 1999;19:966-71.

A case of prenatal diagnosis of Rett syndrome due to germ-line mosaicism. *F. Mari¹, F. Cogliati², R. Caselli¹, I. Longo¹, F. Ariani¹, M. Bruttini¹, I. Meloni¹, K. Schurfeld³, P. Toti³, S. Russo², L. Larizza², G. Hayek⁴, M. Zappella⁴, A. Renieri¹.* 1) Medical Genetics, Molecular Biology Department, University of Siena, Siena, Italy; 2) Laboratory of Molecular Biology, Istituto Auxologico Italiano, Milano, Italy; 3) Department of Anatomy and Pathology, University of Siena, Siena, Italy; 4) Department of Child Neuropsychiatry, Azienda Ospedaliera Senese, Siena.

Rett syndrome is an X-linked neurodevelopmental dominant disorder that almost exclusively affects girls. The vast majority of cases are sporadic and are caused by de novo mutations in MECP2 gene, located in Xq28. Only few familial cases have been reported: in four cases the mother was an asymptomatic carrier and in four cases germ-line mosaicism in the mother was postulated. Due to the above reported cases of germ-line mosaicism we decided to offer prenatal diagnosis to all expectant mothers with a Rett daughter despite the absence of the causative mutation in their blood. We describe here the out-come of the first nine cases of prenatal diagnosis followed by our center. In eight cases (5 females and 3 males) the fetus did not carry the mutation. In one case the female fetus did carry the same mutation as the affected sister. This positive prenatal test strengthens the importance of performing prenatal diagnosis in all cases of Rett syndrome even when the mutation is apparently de novo. Moreover, our results indicate that germ-line mosaicism may be considered for the assessment of recurrence risk during genetic counseling. The couple decided to interrupt the pregnancy and to devolve fetal tissues for research purposes. Taking advantage of the availability of these unique tissues we are performing a morphologic comparison between the Rett fetus and an age-matched control fetus. In addition X-chromosome inactivation studies of different brain areas (hippocampus, cortex, and cerebellum) and other organs are ongoing.

Dandy Walker malformation, aniridia, and cataracts in a 19-week fetus with partial trisomy of chromosome 15. The importance of ophthalmologic pathology in cases of fetal and neonatal demise. *C.S. Rao¹, M.A. Côté-Primac², B. Huang^{1,3}, M. Bocian¹.* 1) Division of Human Genetics, Department of Pediatrics, University of California Irvine Medical Center, Orange, CA; 2) Department of Pathology, University of California Irvine Medical Center, Orange, CA; 3) Genzyme Genetics, Orange, CA.

A 19-week fetus with Dandy Walker malformation (DWM), aniridia, cataracts, and trisomy of chromosome 15pter15q15, is described. Although DWM has been associated with multiple chromosomal defects and Mendelian disorders, chromosome 15 has not been among those reported, nor have DWM and aniridia been reported together in the same individual. Approximately a third of all cases of aniridia are sporadic, and these are often found to have cytogenetically detectable deletions involving 11p13. No locus other than 11p13 has been implicated in aniridia, either sporadic or familial, and PAX6 appears to be the major, if not the only, gene responsible (a report of reanalysis of the family on which mapping of an aniridia locus to 2p was based has excluded linkage to markers on 2p and shown strong indication of linkage to markers in the 11p13 region).

In an unrelated case, aniridia and cataracts were found in a 19-week, cytogenetically normal fetus with other anomalies, including anencephaly and diaphragmatic hernia.

The first case suggests that there are yet undiscovered genes on chromosome 15 which, either alone or through regulation of PAX6, may contribute to the development of aniridia. This case also allows inclusion of partial trisomy 15 among the heterogeneous causes of DWM.

Both cases illustrate the importance of ophthalmologic pathology evaluation of anomalous fetuses and stillborns, especially those with central nervous system abnormalities.

High risk pregnancies in Diamond Blackfan anemia : A survey of 41 pregnancies from the French registry. *L.*

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Diamond Blackfan anemia (DBA) is a rare disorder characterized by red cell aplasia and inconstant congenital anomalies. Few data are available concerning pregnancies in Diamond Blackfan anemia affected women. We report on data from the French Diamond Blackfan anemia registry concerning women aged 25 years or more. Among 25 women (mean age 34.2 years, range 25-46), 14/25 experienced one or several pregnancies (56%). A total of 41 pregnancies occurred, 11 being normal and 30 being complicated (73%), including miscarriages, pre-eclampsia, in utero fetal death, intrauterine growth retardation, pre-term delivery, retro-placental hematoma and fetal malformations. As a result of those 41 pregnancies, only 18 children were born (44%). We did not find any obvious correlation between complicated pregnancies and therapeutic responses, the RPS19 status, or the affected status of the child. Such facts suggest some complications to potentially originate from the affected mother, irrespective of the DBA status of their offspring. In most cases, transfusion requirement and steroid dosage increased during pregnancy. Among the 11 women who did not present any pregnancy, 5 did not desire children for various reasons including their chronic illness, mental retardation (1), infertility (4). Infertility was likely to be treatment related. From this study together with the literature data, we conclude that pregnancies in DBA are at high risk, especially for complications of vascular-placental origin. Such pregnancies should be carefully monitored with a satisfying control of anemia and early introduction of aspirin, especially in cases where a mother experienced a previous complicated pregnancy.

Raine Syndrome Presenting Prenatally as Craniosynostosis: Histologic Findings and Discussion of Pathogenesis.

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Raine syndrome (OMIM #259775) has been classified as a lethal osteosclerotic bone dysplasia with significant craniofacial findings. Here we review a case presenting prenatally at 23 weeks on ultrasound with craniosynostosis with cloverleaf skull, hypertelorism, proptosis, and midfacial hypoplasia. The infant died shortly after birth at 32 weeks gestation. Midsagittal and coronal synostosis was noted. The skull bones were brittle and thick. Radiographs revealed focally exuberant periosteal thickening of long bones and ribs, generalized increased bone density. Coronal sections of the brain revealed multiple foci of calcification, in the white matter, corpus callosum, basal ganglia and internal capsule. The cerebellar leptomeninges showed accumulation of pale basophilic mucinous material within mononuclear histocytes. Sections of rib and femur showed marked thickening of bony trabeculae with central non-mineralized abnormal cartilage precursor material. There was marked osteoblastic activity and periosteal reactive spindle cell proliferation including collections of multi-nucleated giant cells (presumably osteoclasts) apparently ingesting globules of pale basophilic mucoid substance. Sections of the skull showed similar irregular thickening of bony trabeculae. Mucoid material was seen focally. The mucoid substance in the bone is positive for PAS, Alcian blue (pH2.5, but not pH 1.0) and mucicarmine and with acid mucopolysaccharide stain and negative reaction with sulfated mucopolysaccharide stain suggesting that it is cartilaginous precursor material. It is postulated that the substance outside the periosteum is part of an abnormal attempt to produce bone and an attempt to resorb it by giant cells. The appearance of craniosynostosis was secondary to the thick bony trabeculae, periosteal reaction and aggregates of mucoid material and differs histologically from suture fusion of typical craniosynostosis.

Preimplantation genetic diagnosis for DiGeorge/ Velo Cardio Facial Syndrome(DGS/VCFS). A. Aviram-Goldring¹, J. Levron², E. Schiff², T. Litmanovitz¹, D. Gothelf³, M. Frydman¹, B. Farber², B. Goldman¹, O. Israeli¹, G. Barkai¹, J. Dor², S. Rienstein¹. 1) Human genetic Institute, Sheba Medical Ctr, Tel Aviv, Israel; 2) In Vitro Fertilization Unit, Department of Obstetrics and Gynecology, The Chaim Sheba Medical Center, Tel Hashomer and Sackler School of Medicine,; 3) Behavioral Neurogenetics Clinic, Feinberg Child Study Center, Schneider Children's Medical Center of Israel, Petah Tiqwa, Israel; and Sackler School of Medicine, Tel Aviv University, Israel.

Velo-cardio facial syndrome (VCFS) is a genetic syndrome caused by a microdeletion in chromosome 22q11. It is manifested clinically by typical physical manifestations and neuropsychiatric disabilities. Most cases of VCFS are sporadic (1 in 4,000 live births). Familial or inherited cases of VCFS varies from 6% to 28% of the cases. Individuals with VCFS have 50% chances of passing the micro deletion to their offspring. A family was referred for PGD after diagnosis of 22q11 micro-deletion in the mother and in their three years old daughter, and termination of pregnancy of a VCFS fetus. The couple underwent routine in vitro fertilization with biopsy of embryos on day 3. PGD was performed with LSI DiGeorge/VCFS Region probe, 22q13.3 and centromer 18 as control probes (Vysis, Downers Grove, IL). Embryo transfer was possible in two cycles. In the first cycle four embryos were biopsed and only one was normal and transferred. In the second cycle 9 embryos were biopsed. Among them 4 were with the 22q11 micro deletion and five were normal. Two embryos were replaced resulting in ongoing pregnancy. This is the first report of performing PGD in VCFS embryos derived from couple that the mother is a carrier of the diseases with a dominant mode of inheritance. Our preliminary results demonstrate that PGD using FISH analysis is a reliable technique to ensure transfer of normal embryos after in vitro fertilization. We hope that in the near future the crude method of terminating affected pregnancies in carriers of microdeletion traits, will be replaced by pre-selection of normal embryos for replacement by PGD.

CLINICAL AND GENETIC PRENATAL ASPECTS OF THE BOARDING OF THE CERVICAL CYSTIC HYGROM. *M. Baez-Reyes, G. Razo-Aguilera, D. Mayen-Molina.* Department of Genetics. National Institute of Perinatology. Mexico, D.F.

The fetal cystic hygrom associates until 70% to cromosomopatias and the prenatal diagnosis for ultrasound it allows the evaluation of the amniocentesis. The purpose of the study was correlating the phenotype-karyotype prenatally of the cystic hygrom, the clinical evolution and the propose the boarding of the same. Thus, 82 pregnant women were studied with the prenatal diagnosis of fetus with the anomaly detected by ultrasonography. Later on the genetic counseling and acceptance of the patients it was realized the amniocentesis, clinical revision of the newly born and in the event of fetal death, histopatologic study. The gestational age average of the diagnosis was of 23.5 weeks and maternal age of 27.3 years old. Out of 82 cases: 29 (35.3%) presented septal hygroms, 33 cases (40.2%) were non septals and 20 cases (24.3%) with uncertain septation. Fifty three fetus developed hidrops independently of the hygrom type, being for all them letal. The cytogenetics results obtained were: 45 normal (54.8%)(28 female and 17 male) and 32 abnormal (39.0%), being in order of frequency: 21 cases with Monosomy of the "X", 9 cases with regular trisomy 21, 1 case with a traslocation (13;18) and 1 case with one marker. In 5 cases it wasn't possible to realize cytogenetic study because they presented fetal death and not viable tissue. The fetal mortality was present in 56 cases (68.2%). For such reason the ultrasonographic discovery of cystic hygrom is indicative of fetal karyotype, likewise, it is fundamental the pursuit to see the evolution, to determine the pronostic and give real genetic advice.

Semi-quantitative fluorescent PCR (QF-PCR) for rapid aneuploidy screening on amniocentesis and CVS specimens: A blinded prospective trial using material discarded during routine cytogenetic analysis. *L. Brown*¹, *M. Abigania*¹, *D. Warburton*², *S. Brown*¹. 1) Dept OB/GYN, Columbia Univ, New York, NY; 2) Dept. Genetics and Development, Columbia Univ. New York, NY.

Successful aneuploidy screening of prenatal specimens using QF-PCR has been reported. Given that FISH is relatively expensive, a high-throughput technique such as QF-PCR is an attractive alternative. In order to validate QF-PCR for clinical testing, we have set out to determine its reliability in a blinded prospective study in our institution. Rather than obtaining consent from each patient to draw extra amniotic fluid, we have developed a protocol that uses the discarded remains of specimens received for routine cytogenetics. Our PCR protocol is a modification of published methods and consists of 15 markers that are amplified in two separate multiplexed reactions. The 15 loci we have chosen include at least 3 polymorphic markers each on 13, 18, 21 and X as well as amelogenin. PCR products are run on an ABI 310 automated sequencer and are analyzed using Genescan and Genotyper software. Our analysis criteria are based on published methods that have proven to be reliable. Each result is reviewed individually to assess data quality.

Because the study is blinded, we cannot compare QF-PCR results with cytogenetic results until completion of our target of 1000 samples. At this time, we have collected and stored ~900 samples and have completed analysis of 202. Of these, 185 are finalized as normal and 3 as abnormal (1 trisomy 21 and two trisomy 18). Six samples (3%) could not be analyzed due to maternal contamination and another seven were not fully informative despite the use of additional markers. Thus far, our data support the feasibility of QF-PCR for aneuploidy screening in prenatal samples; however, the proportion of samples that cannot be assessed is apparently greater than that reported in other series.

Rapid prenatal screening using FISH in uncultured amniocytes. *V. Catala, E. Cuatrecasas, C. Garrido, L. Vila, T. Escabias, A. Ramos, A. Seres-Santamaria.* Prenatal Genetics, Barcelona, Catalonia, Spain.

Fluorescence in situ hybridization (FISH) allows the evaluation of chromosome copy number in interphase cells. This characteristic gives FISH a great potential for the study of amniotic fluid cells in prenatal diagnosis. The major indications in prenatal diagnosis, advanced maternal age, fetal malformation detected by ultrasonography, increased risk of trisomy estimated by maternal serum screening and maternal anxiety demands a rapid and accurate result. Classical cytogenetic analysis of amniocytes is the best technique to detect numerical and structural chromosome aberrations after culture of amniotic fluid cells, but this study is not completed in less than a week. FISH in uncultured amniotic fluid cells permits the detection of most common aneuploidies in less than 24 h after amniocentesis. We present a prospective study of 4121 uncultured amniotic fluid (AF) samples by FISH, using commercial probes specific for 13, 18, 21, X and Y human chromosomes. FISH studies in AF cells were performed in our laboratory as an adjunctive of conventional cytogenetic studies using AneuVysion Test Kit (Vysis). Parallel cultures from each sample were grown and standard cytogenetics results were informed at the end of the study. We detected 2.4% of autosomal trisomies and 0.5% of sex chromosomes aneuploidy. Our results support previous studies reporting FISH in uncultured AF cells is a feasible method to assess the most common aneuploidies observed in newborns. FISH has to be considered as an adjunctive method to classical karyotyping, however FISH allows an accurate result in less than 24h, and a rapid result is very important in prenatal diagnosis.

Exocervically retrieved trophoblast cells at 5-12 gestational weeks: a new screening method for Down syndrome?

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Currently, clinically available methods for prenatal chromosomal diagnosis (amniocentesis and cvs) are invasive, risky and costly. Screening techniques are cumbersome and carry a false negative rate of 20%. We assessed the feasibility of utilizing exocervically retrieved extra villous trophoblast cells for screening for chromosome 21-aneuploidy and gender determination. **Methods-** Exocervical sample was obtained from the external cervical os of pregnant women using a cytobrush. Eight slides were prepared using a cytopspin centrifuge. Immuno-histochemistry using HLA-G and other anti trophoblast antibodies. The marked cells were identified and scored. FISH for X and Y was applied and the previously marked cells were returned to for diagnosis. FISH for chromosome 21 was applied later to the same cells. **Results-** Initially, samples were taken from 227 women prior to pregnancy termination. In 195 (86%) were trophoblast cells detected. Gender diagnosis concurred with that of the placental in 186 (95%). Subsequently 130 on-going pregnancies were screened. Trophoblast cells were detected in 112 (86%). Sex was correctly determined in 100 (89%). There was 1 case of trisomy 21 that was correctly diagnosed. There were 2 cases of missed abortions both initially diagnosed to be XY. Placental tissue karyotyping revealed both of them to be mosaics, 46,XY/45,XO and 47,XXY/46,XY. **Conclusion-** this simple and inexpensive procedure may have the potential to replace currently available non-invasive screening techniques for Down syndrome.

Result of chromosome abnormalities in Korean population identified by triple-marker testing as screen positive for Down and Edward syndrome. J. Kim. Department of cytogenetics, GreenCross Reference Laboratory, Korea.

Background:Second-trimester serum screening can detect 60-65% of cases of Down syndrome and 60% of Edward syndrome. Previous studies reported a high frequency of chromosomal abnormalities other than trisomy 21 and trisomy 18 in the screen positive population. But rate and type of other chromosome abnormalities detected was inconsistent in each study and different between the race. This study was to determine the incidence of chromosomal abnormalities in Korean women receiving chromosome analysis after screen positive result by using maternal age and serum triple-marker testing(FP, HCG, uE₃). **Methods:**A total of 49,806 pregnant women between 15 and 23 gestational weeks received second-trimester prenatal screening. By use of a 1 : 270 Down syndrome and 1 : 100 Edward syndrome risk cutoff value, 2,116 and 196 pregnant women were screen positive for Down syndrome(screen positive rate 4.2%) and positive for Edward syndrome(screen positive rate 0.04%). Prenatal chromosomal analysis in amniotic fluid were performed for 1893(89.5%) of Down positive and 140(71.4%) of Edward positive women. **Results:**Forty-one trisomy 21(2.2%) and 42 other chromosomal abnormalities(2.2%) were identified in the Down screen-positive group. Other chromosomal abnormalities included 11 balanced translocation, 10 Robertsonian translocation, 8 inversion, 7 sex chromosome abnormality, 2 deletion, 1 isochromosome 22, 1 trisomy 20, 1 de novo marker, 1 derivative 15. Seven sex chromosome abnormalities included 4 45,X, 2 47,XYY and 1 47,XXY. For Edward screen-positive pregnancy, 9 trisomy 18(6.4%) and 1 trisomy 9(0.7%) were detected. **Conclusion:**Our study suggest that 4.4% of Down positive pregnancy and 7.1% of Edward positive pregnancy will show chromosome abnormality in Korean. Positive Down screening result reflected relatively high probability of other abnormalities except trisomy 21. The type of other chromosome abnormalities in Down screen positive group showed difference compared to other studies and the rate of chromosome abnormalities was higher than in other Asian population. Edward screen positive group did showed low frequency of other chromosome abnormalities except trisomy 18.

The prenatal diagnosis of a fetus with de novo ring chromosome 11. *J.Y. Park¹, S.Y. Park¹, M.H. Lee¹, E.H. Cho¹, B.Y. Lee¹, Y.W. Lee¹, H.M. Ryu^{1,2}.* 1) Laboratory of Medical Genetics, Samsung Cheil Hosp, Seoul, Seoul, Korea; 2) Department of Obstetrics and Gynecology, Sungkyunkwan University School of Medicine, Seoul, Korea.

We report a de novo ring chromosome 11 in prenatal diagnosis. A 36-year-old pregnant woman was referred for amniocentesis at 19.5 weeks gestation because of an advanced maternal age and increased risk for Edward syndrome in maternal serum screening test. Cytogenetic analysis of the cultured amniotic fluid cells revealed mosaicism for ring chromosome 11; 46,XX,r(11)[65]/45,XX,-11[16]/46,XX[34]. Both parents had a normal karyotype. A targeted ultrasound showed no significant abnormalities other than intrauterine growth restriction(IUGR). Cordocentesis performed to characterize of ring chromosome by high resolution and reverse banding. In this culture no normal karyotype was observed ; 46,XX,r(11)(p15.5q24.2)[229]/45,XX,-11[15]. The deletion of subtelomeric region in the ring chromosome was detected by fluorescent in situ hybridization(FISH). After genetic counseling the parents did not want to continue the pregnancy. Autopsy showed significant IUGR and rocker bottom feet. To the best of our knowledge, ring chromosome 11 is a very rare case and especially only one case which de novo ring chromosome 11 in prenatal diagnosis have been reported.

Case report of the prenatal diagnosis & management of fetal achondroplasia & Down Syndrome. *T. Prosen*^{1, 2}, *M. Kolthoff*^d. 1) Dept OB/GYN Reprod Sci, Magee Womens Hosp, Pittsburgh, PA; 2) Fetal Diagnosis & Treatment Center, Magee Womens Hosp, Pittsburgh, PA.

We report on a case of the prenatal diagnosis and management of fetal achondroplasia and Down Syndrome. A 36 year-old primagravida with maternal achondroplasia presented at 20 weeks gestational age for consultation. She was found to have an elevated risk for Down Syndrome (1 in 12) by second trimester multiple marker screening. A genetic sonogram was performed that showed mild fetal pyelectasis, increasing the risk to 1 in 6. The patient declined amniocentesis. At 27 weeks, an ultrasound for fetal biometry revealed fetal long bone measurements to be less than the 5th percentile for gestational age, consistent with fetal achondroplasia. In addition, no nasal bone was identified. At 30 weeks and 6 days, the patient presented for routine follow-up. At that time, ultrasound revealed fetal hydrops (pleural and pericardial effusions, ascites and anasarca), polyhydramnios, and abnormal umbilical artery Doppler velocimetry studies. The patient received 48 hours of beta-methasone therapy followed by a primary cesarean section for fetal hydrops and maternal achondroplasia. The premature male infant was stabilized in the neonatal intensive care unit. Neonatal survey showed characteristics of both achondroplasia and Down Syndrome. The infant exhibited trident hands, rhizomelic limb shortening, large head with frontal bossing, down-slanting eyes, and single palmar creases. The infant's karyotype was found to be 47, XY, +21. The diagnosis of achondroplasia was confirmed by the presence of the G1138A gene mutation in the FGFR3 gene. To our knowledge, this is the first reported case of fetal Down Syndrome and achondroplasia detected and managed prenatally. The combination of a mendelian disease with a chromosomal abnormality creates a complex scenario for prenatal diagnosis and management.

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CYTOGENETICS PRENATAL DIAGNOSIS IN FETAL FLUIDS. *G. Razo-Aguilera, R. Baez-Reyes, JL. Saucedo, J. Zavaleta, D. Mayen-Molina, R. Garcia-Cavazos.* Department of Genetics. National Institute of Perinatology. Mexico, City.

The invasive techniques traditionally used to obtain fetal cells for chromosome analysis are chorionic villus sampling, amniocentesis and cordocentesis. However, in some cases those methods are difficult to perform, specially in the presence of structural defects in the fetus, advanced gestational age or oligoamnios, so an alternative method is required. In those cases with fetal structural defects detected by high resolution ultrasound where traditional invasive procedures are difficult to perform, we could offer to pregnant women somatocentesis. We performed fifty nine cases of somatocentesis in fetal cells from cystic hygroma, urine and pleural and ascitic fluids. The cytogenetic results in seven samples: five from cystic hygroma and two from urine. The 13 abnormal karyotypes include: 11 from cystic hygroma: 10 cases with 45,X; 1 case with 46,XY,der(13)t(13;18). The somatocentesis is a good alternative method to obtain fetal cells in cases where traditional methods represent technical difficulties and with specific modifications in cytogenetic technique according to the cell type a karyotype with good band resolution can be obtained.

First trimester Down syndrome screening: high detection rate for trisomy 21, but poor performance in structural abnormalities. *M. Ryyanen*¹, *A. Rissanen*², *J. Taskila*¹, *P. Laitinen*¹, *S. Heinonen*², *M. Suonpaa*³. 1) Dept OB/GYN, and Clin. Chemistry Oulu Univ Hosp, Oulu, OYS, Finland; 2) Dept OB/GYN, Kuopio Univ Hosp, Kuopio, Finland; 3) PerkinelmerWallac, Turku Finland.

Objective: To evaluate whether first trimester screening markers are altered in pregnancies affected both by other chromosomal defects than trisomy 21 and structural anomalies and are these pregnancies possible to detect by combined ultrasound and biochemical screening test. **Methods:** 4776 singleton pregnancies underwent first trimester screening between 10th and 13th gestation weeks. N women were screened using a combination of maternal serum free hCG, PAPP-A and nuchal translucency and N women with first trimester biochemistry without ultrasound. **Results:** Using a fixed cut-off rate of 1:25 for Down's syndrome, the detection rate of trisomy 21, 18 and 13 were 92%, 67% and 0%, respectively. All open defects, 87% of cardiac abnormalities and other minor defects were not detected in first trimester screening. Majority of these structural abnormalities occurred in women under 35 y of age. **Conclusion:** First trimester Down syndrome screening is effective in trisomy screening, but its performance in structural abnormalities is low, when used as a part of routine clinical practice. We conclude that it is too early to drop second trimester screening ultrasound entirely from antenatal care programs if a high detection rate is to be achieved also in structural defects.

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Invasive Trophoblast Antigen (ITA) Clinical Utility in First and Second Trimester Prenatal Screening for Trisomy 21 and Other Chromosomal Abnormalities: Preliminary Report of a Prospective Study in Germany. *U. Sancken, D. Taube.* Inst Human Genetics, Univ Goettingen, Goettingen, Germany.

ITA is a hyperglycosylated form of human chorionic gonadotropin (hCG) found in elevated levels of blood and urine during pregnancy. Retrospective studies indicate that ITA is useful in prenatal screening for Down syndrome. We have conducted a prospective observational study to confirm this utility. Serum samples were collected from women undergoing routine prenatal care (2,927 in the first trimester and 2,233 in the second). From 7244 available cases we considered only those with gestational dating by early fetal crown-rump length measurement. Maternal ages ranged from 15 to 46 years (average: 30 years). ITA levels were determined for all samples, and outcome information was based on karyotype or phenotype following birth. Outcome information, based on 88% of the women, has identified 26 cases of trisomy 21 (Down syndrome), 7 cases of trisomy 18, 1 trisomy 13, 1 trisomy 22, 7 triploid cases, and several other chromosomal abnormalities (eg, XO, XXY, translocations). Serum ITA levels were very low (all but one below the 5th centile) in the trisomy 18, trisomy 13, and triploidy cases. ITA levels were greater than 1.0 multiple of the median (MoM) in all cases of trisomy 21 and 22. In trisomy 21 cases, the median MoM was 1.8 in the first trimester and 3.2 in the second trimester. More importantly, 33% of the Down syndrome cases had ITA levels above the 95th centile in the first trimester. In the second trimester, levels exceeded the 95th centile in 50% of the Down syndrome cases. Thus, ITA is a useful screening marker for fetal chromosomal abnormalities in both the first and second trimesters. For Down syndrome screening, ITA is most useful in the second trimester.

Variation in the Decision to Terminate Pregnancy in the Setting of an Abnormal Karyotype with Uncertain

Significance. *B.L. Shaffer*¹, *A.B. Caughey*¹, *P.D. Cotter*², *M.E. Norton*¹. 1) Dept OB/GYN, Univ Cal, San Francisco, San Francisco, CA; 2) Dept of Pediatrics, Children's Hospital, Oakland, CA.

Approximately 2-3% of karyotypes from prenatal diagnosis are abnormal and a significant proportion are of uncertain significance. An unclear prognosis poses a unique clinical situation, how much "risk" of phenotypic abnormality is too much?

To examine predictors of termination of pregnancy, we performed a retrospective cohort study at a prenatal diagnosis unit from 1984-2003. Patients with karyotypic abnormalities of marker or mosaic, balanced and unbalanced rearrangements were categorized in three groups of similar prognosis. We examined chromosomal abnormality, age, ethnicity, parity, and previous abortion as predictors of the decision to terminate.

There were 419 abnormal fetuses and the termination rate was 20%. Each group was different: 6.5% of patients with balanced rearrangements, 61% with unbalanced abnormalities, and 29% with marker/mosaic karyotypes terminated ($p < 0.001$, ²). Among patients with balanced rearrangements, those with inherited abnormalities had a termination rate of 3% versus 13% in those with a newly diagnosed balanced rearrangement ($p = 0.003$, ²). There was no difference in those with an unbalanced rearrangement, with the inherited group's rate 63% and the newly diagnosed group's rate 59%. In multivariate analysis, karyotypic abnormality was a significant predictor of termination, but age, ethnicity, parity, and previous abortion were not.

In patients with an abnormal karyotype of uncertain significance, termination rates increased with increasing chance of phenotypic abnormality. In contrast to reports of termination in common aneuploidies, we found no differences between women of different ethnicity or ages in the decision to terminate. Perhaps, when families are faced with an uncommon diagnosis of uncertain significance they have fewer preconceived biases, and their decision is unrelated to ethnicity, age, or previous reproductive choice.

Outcome of Prenatally Diagnosed Individuals with 45,X/46,XX and 45,X/46,XY Mosaicism. *L. Tan, P. Eydoux, B. McGillivray.* Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada.

Although prenatally diagnosed 45,X/46,XX and 45,X/46,XY mosaicism have been shown to have a milder prognosis when compared to non-mosaic 45,X, such results present a genetic counselling dilemma. The literature is primarily retrospective, consisting of postnatally diagnosed cases while the small number of prospective studies is limited to a short follow-up period. In this study, we evaluated the long term outcome of prenatally diagnosed cases with 45,X/46,XX mosaicism (n=12) and 45,X/46,XY mosaicism (n=14). Upon testing, mosaicism was not confirmed postnatally in 6 of the 12 prenatally diagnosed cases with 45,X/46,XX mosaicism. 3 cases had abnormal prenatal ultrasound findings. The abnormalities seen in 2 cases were not associated with 45,X/46,XX mosaicism. In the absence of prenatal ultrasound abnormalities, all were clinically normal. Mosaicism was not confirmed postnatally in 5 of the 14 prenatally diagnosed cases with 45,X/46,XY mosaicism. Postnatal cytogenetic analysis of 1 case determined an isodicentric Y chromosome previously reported as a normal Y chromosome. 12 cases were examined and found to be phenotypically normal male with no Turner syndrome stigmata. 2 cases with prenatal ultrasound abnormalities were prenatally diagnosed with non-mosaic 45,X. One was phenotypically female with streak gonads and gonadoblastoma while the other had ambiguous genitalia at birth. Prenatal counselling for 45,X/46,XX and 45,X/46,XY mosaicism in the absence of prenatal ultrasound abnormalities should take into account the expectation of a milder phenotype than that of patients ascertained postnatally. Postnatal confirmation of prenatal cytogenetic diagnosis is very important because a significant number of prenatally diagnosed mosaicism could not be documented postnatally. The present data suggest the need for follow up ultrasound examination of fetal genitalia and thorough cytogenetic and molecular analysis for Y chromosome material to rule out the possibility of 45,X/46,XY mosaicism in prenatally diagnosed cases with non-mosaic 45,X. We found no correlation between the proportion of 45,X cells in amniotic fluid and the presence or degree of abnormalities.

Prenatal detection of full and mosaic trisomy 9. Report of five new cases and review of the literature. *L. Turner¹, S. Langlois¹, L. Arbour¹, S. Lewis¹, M. van Allen¹, T. Pantar², G. Taylor², S. Yong¹*. 1) Department of Medical Genetics, University of British Columbia, Vancouver, Canada; 2) Department of Pathology, University of British Columbia, Vancouver, Canada. Children's and Women's Health Centre of British Columbia.

Trisomy 9 is a relatively uncommon chromosome abnormality that may be present in a mosaic or nonmosaic state. It involves a distinct pattern of dysmorphic features and congenital anomalies. Common facial findings include a sloping forehead, low set ears, and beaked nose. Other anomalies include heart defects, most commonly ventricular septal defects, renal and CNS anomalies. We report five new cases of trisomy 9 (4/5 cases with putative full trisomy 9 and 1/5 cases with mosaic trisomy 9). All cases were diagnosed prenatally. Two cases were ascertained due to a positive maternal serum screen for trisomy 18, two cases due to an abnormal fetal ultrasound, and one case because of an abnormal karyotype on amniocentesis, performed for maternal age indication.

We report the ultrasound (4/5) and embryopathology (4/5) findings of our cases in order to add to the described phenotype of trisomy 9. All cases in which a detailed ultrasound was done had ultrasound abnormalities. These abnormalities included various combinations of CNS (3/4), cardiac (2/4), thoracic (2/4), renal (2/4) and hand and foot (2/4) abnormalities. Additional findings at embryopathology were craniofacial dysmorphism (4/4), malrotation (1/4), coloboma (1/4), and uterine abnormalities (3/4). Anomalies seen which have been rarely reported in the literature included diaphragmatic eventration (2/3) and uterine abnormalities in three out of four females cases (didelphic uterus (2) and bicornuate uterus (1)). We describe our findings and also review published cases of prenatally detected trisomy 9. Our cases and those reported in the literature emphasize that fetuses with trisomy 9 have multiple anomalies that can be detected prenatally by ultrasound.

Blood lymphocyte chimerism associated with in vitro fertilization and monozygotic twinning. C.A. Williams¹, M.R. Wallace^{1,2}, K.C. Drury³, S. Kipersztok³, R.K. Edwards⁴, M.J. Haller⁵, D.A. Schatz⁵, J.H. Silverstein⁵, B.A. Gray¹, R.T. Zori¹. 1) Dept Pediatrics/Genetics, Univ Florida, Gainesville, FL; 2) Department of Molecular Genetics and Microbiology, Univ Florida, Gainesville, FL; 3) IVF Program and Laboratory, Division of Reproductive Endocrinology and Infertility, Univ Florida, Gainesville, FL; 4) Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Univ Florida, Gainesville, FL; 5) Division of Endocrinology, Univ Florida, Gainesville, FL.

We report on dizygotic twins, conceived by IVF and intracytoplasmic sperm injection (ICSI) with assisted hatching, who each had a mixture of 46,XX and 46,XY cells in blood lymphocytes. The female twin had mild ambiguous genitalia but further study revealed anatomically normal reproductive anatomy. Chromosome and fluorescence in situ hybridization (FISH) studies of buccal, skin and ovarian tissue were normal as were buccal tissue DNA studies. Fetal ultrasound and fetal membrane pathology were consistent with a monozygotic, diamniotic placenta (MCDAP). These twins thus have blood chimerism but are not chimeric in the other tissues studied. The mechanism for the chimerism could be due to either placental vascular anastomoses (after the development of the hematoblast stem cells) or due to an admixture of trophoblast cells during early blastocyst development. Such trophoblast cell admixtures would be restricted to the extra embryonic tissues so that general physical development in the fetus is normal and without somatic cell chimerism. This case in combination with others previously reported suggests that in IVF conceptions, the prevalence of blood chimerism associated with twinning, and the occurrence of DZ twinning associated with MCDAP, is higher than previously thought.

Endometriosis and microchimerism: further expanding the association of fetomaternal cell trafficking and disease in women. *D.H. Cha^{1, 2}, K. Khosrotehrani¹, S.W. Lee², D.W. Bianchi¹, K.L. Johnson¹.* 1) Division of Genetics, Department of Pediatrics, Tufts-New England Medical Center, Boston, MA; 2) Department of Obstetrics and Gynecology, Kangnam CHA Hospital, Pochon CHA University, Seoul, Korea.

Objective. The use of fetal cells in maternal blood for genetic diagnosis has led to an appreciation of the persistence of fetal cells post-partum, creating a state of microchimerism. To date, microchimerism has been linked to some autoimmune disorders, tumors and infectious disease in women. Our objective was to determine whether fetal cell microchimerism is associated with the pathogenesis or progression of endometriosis.

Methods. Ovarian and tubal tissue were obtained from 5 women with endometriosis who had at least 1 live-born son. Control tissue was obtained from 5 women with endometriosis and no male births. Tissue sections were analyzed by FISH for the presence of male fetal cells, defined by the presence of X and Y chromosome signals. Immunolabeling using an epithelial cell marker (cytokeratin) and the leukocyte common antigen (CD45) was performed to determine the phenotype of the presumed fetal cells.

Results. Male cells were found in tissue from all five endometriosis patients with a history of male births. No male cells were found in tissue specimens from controls. Immunolabeling studies showed that 4.4% of XY cells (4/90) expressed cytokeratin and 63.3% of XY cells (57/90) expressed CD45.

Conclusions. Endometriosis may be associated with persistent fetal cells resulting from fetomaternal cell trafficking, with leukocytes being the predominant microchimeric cell type present. As previous experiments in our laboratory showed a lack of cytokeratin-positive cells in umbilical cord blood, it is also possible that some level of differentiation of pregnancy-associated progenitor cells has occurred in these women. These results further expand the potential relationship between fetal cell microchimerism and disease in women.

Cytogenetic and Molecular Analysis in male infertility. *R. Dada*¹, *N.P. Gupta*³, *K. Kucheria*². 1) Anatomy, AIIMS, New Delhi, India; 2) Anatomy and Genetics, AIIMS, New Delhi, India; 3) Urology, AIIMS, New Delhi, India.

In the last few years considerable progress has been made towards understanding sperm physiology and the biology of gamete interaction to understand the pathophysiology of male infertility. Three regions on Yq (AZFa, AZFb and AZFc) are critical for spermatogenesis. Deletions in AZF region can cause severe spermatogenic defects. These loci act at different stages of germ cell development and deletion of each loci result in a characteristic phenotype. In order to study frequency of AZF microdeletion and genotype phenotype correlation in infertile this study was undertaken. Idiopathic oligozoospermic and azoospermic cases were included in this study. Cytogenetic and semen analysis was done in each case. Testicular FNAC was collected whenever possible. On cytogenetic analysis Klinefelter Syndrome (KFS) was the major cytogenetic anomaly followed by mosaic KF, variant Klinefelter, and cases with other numerical and structural cytogenetic abnormalities. In cytogenetically normal cases (n=189) microdeletion analysis was done. Microdeletions spanning the AZF loci were found in a 6.3 percent cases (12 cases). Microdeletions were also found in one case with KFS which have not been reported yet. The FSH and LH levels were elevated in 8 of these cases with Yq microdeletion and in 4 cases were in the normal range. Thus raised FSH levels does not indicate presence of Yq microdeletion and low FSH does not indicate absence of Yq microdeletion. Thus Testicular FNAC is a must in all these cases. In one cases with Yq microdeletion cytogenetic analysis showed 46,XY and 45,XO mosaicism. Therefore presence of cytogenetic anomalies and detection of Yq microdeletions encompassing the AZF loci determines the prognosis and management of these infertile cases. All infertile men with normal or raised FSH levels should be counselled to go in for Yq microdeletion analysis. Also these infertile men with normal or low semen parameters have DNA damage secondary to other factors like oxidative stress and thus should be counselled of offspring being more prone to malignancies and having a much more severe testicular phenotype in addition to being infertile.

A rare case of possible 3:1 meiotic segregation and subsequent interchange trisomy 18 in a fetus whose mother carries a t(12;18)(p13.1;q21.1) translocation. *C.M. Coffeen¹, S. Bhatt¹, M. Thangavelu¹, D.B. Rogers¹, L.D. Platt^{2,3}.*
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A balanced reciprocal translocation can result in a variety of unbalanced products during gamete formation, depending on the segregation outcome. In meiosis I, the chromosomes involved in a translocation form a quadrivalent configuration to ensure maximum pairing of the homologous segments. The homologues subsequently segregate from each other in one of three possible ways: 2:2, 3:1 or 4:0 segregation. This meiotic behavior increases the risk for both structural rearrangements and whole chromosome aneuploidy in the offspring of carriers. We present the case of a 36 year-old G8 P4 SAb2 TAb1 woman, referred for genetic counseling at 10.4 weeks gestation for advanced maternal age and due to her karyotype of 46,XX,t(12;18)(p13.1;q21.1). Ultrasound a week later showed an increased nuchal translucency with total body edema, pleural and pericardial effusion, and an omphalocele. Cytogenetic analysis on CVS revealed an abnormal male karyotype [47,XY,t(12;18)(p13.1;q21.1)mat,+18], consistent with the diagnosis of trisomy 18. Given the poor prognosis, the patient elected to proceed with pregnancy termination. Our case represents a rare case of trisomy 18 known as interchange trisomy, which occurs secondary to 3:1 meiotic segregation of the patient's translocation during gamete formation. 3:1 segregation is most likely to occur when one of the whole chromosomes (normal or derivative) of the quadrivalent is small. In our case, both the normal chromosome 18 and der(18)t(12;18) are small when compared to the normal chromosome 12 and the der(12)t(12;18). Therefore, the origin of the additional chromosome 18, in this case, is most likely due to a 3:1 segregation in the mother, who is a carrier of the t(12;18). This case re-enforces the importance of considering the principles of segregation in estimating risk assessment to the offspring of translocation carriers.

Chromosomal abnormalities in 2078 infertile couples referred for assisted reproductive techniques (ART). G. Palka^{1,2}, P. Guanciali-Franchi^{1,2}, E. Clementini³, G.M. Tiboni³, C. Palka⁴, I. Iezzi^{1,2}, A. Di Tecco², E. Morizio², D. Fantasia^{1,2}, M. Marino¹, L. Stuppia¹, G. Calabrese^{1,2}, B. Dallapiccola⁴. 1) Dept Sci Biomediche, Univ G D'Annunzio, Chieti Scalo, Italy; 2) Genetica Umana, Ospedale Civile, Pescara, Italy; 3) Dept Medicina e Sci Invecchiamento/Ostetricia & Ginecol, Univ G D'Annunzio, Chieti Scalo, Italy; 4) CSS-Inst Mendel, Rome, Italy.

BACKGROUND: In this study the prevalence of karyotype changes and Yq11 microdeletions in a sample of couples referred for infertility was determined. **METHODS:** The studied population accounted 2078 couples ongoing treatment by either in-vitro fertilization (IVF) or intra cytoplasmic sperm injection (ICSI), in which both partners presented without any obvious phenotype related to some chromosomal changes. Cytogenetic investigation was performed on peripheral lymphocytes of both partners of each couple using GTG-banding technique. All the oligo/azoospermic men with normal karyotype were further investigated by PCR for Yq11 microdeletions. **RESULTS:** Eighty-two out of 2078 couples (3.95%) resulted to have one partner carrying a chromosomal change associated to poor gestation outcome or infertility. The frequency of the anomalies between sexes was similar, namely 42 and 40 anomalies among men and women, respectively. Ten out of 178 males studied for abnormal spermatogenesis showed Yq11 microdeletion (5.6 %). **CONCLUSIONS:** Chromosomal anomalies as translocations, inversions and complete gonosomal aneuploidies, which all may affect the reproductive fitness, recurred with a frequency greater than that reported in a large population of unselected newborns. Present data suggest that cytogenetic evaluation of couples candidate to ART is advisable, in order to identify couples at risk for poor gestational outcome.

Risk factors in abdominal wall defects. *B. Dott, M.P. Roth, C. Stoll.* Genetique Medicale, Faculte de Medecine, Strasbourg, France.

The aim of this study was to describe the prevalence at birth of two abdominal wall defects (AWD), omphalocele and gastroschisis and to identify possible etiologic factors. The AWD came from 320,984 consecutive births of known outcome registered in the registry of congenital malformations of Strasbourg for the period 1979 to 2002. Request information on the child, the pregnancy, the parents and the family was obtained for cases and for controls. Hundred twenty one cases with AWD were analysed, 55.4 % were omphalocele and 44.6 % were gastroschisis. The mean prevalence rate for omphalocele was 2.18 per 10000 and for gastroschisis 1.76 per 10000. Associated malformations were found in 74.6 % of omphalocele compared with 53.7 % of gastroschisis; 28.3 % of fetuses with omphalocele had an abnormal karyotype, 44.7 % had a recognizable syndrome, association or an unspecified malformation pattern; 51.8 % of fetuses with gastroschisis had additional malformations that were not of chromosomal origin, but 1 case. Antenatal ultrasound examination was able to detect 45 (67.2 %) cases of omphaloceles and 31 (57.4 %) cases of gastroschisis. In 35 (52.2 %) cases of omphalocele and in 8 (14.8 %) cases of gastroschisis parents opted for termination of pregnancy. The overall survival rate was 16 (23.9 %) for omphalocele and 35 (64.8 %) for gastroschisis. Weight, length and head circumference at birth of infants with AWD were less than those of controls. The weight of placenta of infants with AWD was not different from the weight of placenta of controls. Gastroschisis was associated with significantly younger maternal age than omphalocele. Pregnancies with AWD were more often complicated by threatened abortion, oligohydramnios and polyhydramnios.

Fertility potential in the cryptorchid male using spermatozoal gene expression microarray analysis. M.T.

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Cryptorchidism, failure of testicular descent, is associated with male infertility. Previously thought to be transcriptionally dormant, recent studies suggest spermatozoal RNA as a measure of past events during spermatogenesis. Using microarray analysis, we sought to identify significant differential gene expression patterns between adolescent males with a history of corrected cryptorchidism and normal control patients. Ejaculates were collected and a semen analysis performed on 10 control patients (normal urogenital history/exam and semen analysis by WHO criteria) and 5 cryptorchid male (3 bilateral, 2 unilateral). Motile sperm were separated from round cells and nonmotile sperm by two rounds of Percoll centrifugation, the total RNA extracted and verified using sperm-specific RT-PCR. Biotin-labeled amplified RNA was hybridized to Affymetrix Human Genome Focus Arrays, according to the Affymetrix protocol. To identify genes that are differentially expressed between sperm from cryptorchid and control patients, permutation *t* test was performed. Median semen volume was not significantly different between control and cryptorchid patients (2.1 vs 3.0 cc, $p=0.56$). Median sperm density was markedly decreased in the cryptorchid group (94 versus 10 million/cc, $p=0.06$). From the microarray expression data, we identified 91 genes/ESTs differentially expressed (permutation $p < 0.01$) between the two groups. Of these, 55 genes were under-expressed in the cryptorchid samples including a testis-specific gene (Tpx-1) involved in spermatogenic-Sertoli cell adhesion. An epididymal-specific protein (WFDC2) involved with spermatogenesis was found to be significantly over expressed in the cryptorchid patients, as well. Using microarray analysis we have identified a set of genes which are significantly under- and over-expressed in males with a history of cryptorchidism compared to normal controls. Verification with real-time PCR will aid in gaining insight into the global genetic changes that occur with cryptorchidism.

A NEWBORN BABY PRESENTED WITH SHORT EXTREMITY, THROMBUS AND MTHFR C677T

MUTATION. *N. Ozbek¹, F.B. Atac², E. Ozyurek¹, A. Tarcan¹, H. Verdi², F.I. Sahin², B. Gurakan¹*. 1) Pediatrics, Baskent University School of Medicine, Ankara-Turkey; 2) Medical Biology and Genetics, Baskent University School Of Medicine, Ankara-Turkey.

Hyperhomocysteinemia is a known risk factor for cerebrovascular, peripheral vascular, coronary heart diseases and thrombosis. Heterozygosity and/or homozygosity for mutations in the enzymes involved in homocysteine metabolism may confer an increased risk for thrombosis by leading to hyperhomocysteinemia. A common mutation in methylenetetrahydrofolate reductase (MTHFR) C677T has been known to result in increased plasma homocysteine levels thus causing a predisposition to thrombosis. We hereby report a newborn with thrombosis who is heterozygous for MTHFR C677T. The newborn was a term baby presented with right lower extremity 2 cm shorter than the left. His mother was homozygous for MTHFR C677T mutation. Thrombosis involving right iliofemoral artery was shown immediately after birth. His arterial occlusion was dilated by balloon angioplasty and patent flow was established. Anticoagulation was started with low molecular weight heparin. However, he died with sudden infant death at home when he was 28 days old. No etiologic factor was figured out except heterozygous MTHFR C677T mutation. We exclaim that the thrombotic process in this particular case has been started in utero. Despite he did well with antithrombotic treatment, sudden infant death may be induced by a fatal thrombotic problem yet not proven by postmortem study due to rejection of consent.

First trimester ultrasound diagnosis of lethal multiple pterygium syndrome. *J. Pierre-Louis¹, K. Fong², M. Gundogan², S. Keating³, D. Chitayat¹.* 1) Prenatal Diagnosis Medical Genetics; 2) Departments of Diagnostic Imaging; 3) Laboratory Medicine & Pathobiology, Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada.

Lethal multiple pterygium syndrome (LMPS) is a rare autosomal recessive condition, with a recurrence risk of 25 percent. The gene/genes causing this likely genetically heterogeneous condition have not yet been delineated. We report a first trimester prenatal diagnosis of LMPS. A 38-year-old G5P2SA1SB2L1 woman was seen at 12 weeks gestation. The non-consanguineous, Ashkenazi-Jewish couple's first pregnancy resulted in spontaneous miscarriage. The second pregnancy resulted in dizygotic twins. Detailed ultrasound performed at 18 weeks gestation showed cystic hygroma, webbing and contractures of the knees and elbows, with poor movements in one twin. This twin died at 24 weeks gestation. Autopsy showed severe maceration, dysmorphic facial features, nuchal thickening, multiple flexion contractures and pterygia, kyphoscoliosis, rocker bottom feet, hypoplastic aortic arch, hypoplastic lungs and ambiguous genitalia. These findings were consistent with LMPS. The remaining twin had no apparent abnormality and was delivered at 31.5 weeks following PROM. The couple's third pregnancy had decreased fetal movements on the 24 weeks fetal ultrasound and intrauterine death occurred at 27.5 weeks gestation. Autopsy revealed a female fetus with the same findings as the previous fetus, consistent with LMPS. The patient subsequently had a ruptured ectopic pregnancy. In the couple's present pregnancy, fetal ultrasound at 12 weeks showed a markedly increased nuchal translucency and bilateral flexion deformities of the elbows, wrists, hands and knees. No limb movements were observed during the entire 30-minute examination. The parents requested termination of pregnancy by D&C. Pathology of the intact fetal parts showed flexion deformity of the right elbow with a cutaneous web, contracture deformity and ulnar deviation of the right wrist, and a cleft palate. These findings confirmed LMPS. Thus, first trimester fetal sonography is a practical and reliable method for early prenatal diagnosis of LMPS.

Descriptive epidemiology of birth defects in Malaysian births: a population-based study. *M.K. Thong¹, J. Ho², N. Noor Khatijah³*. 1) Dept Pediatrics, University of Malaya Medical Ctr, Kuala Lumpur, Malaysia; 2) Perak College of Medicine, Ipoh, Malaysia; 3) Hospital Ipoh, Perak, Malaysia.

Data on birth defects from population-based studies originating from developing countries are lacking. The objectives of this study are to determine the epidemiology of birth defects and to identify risk factors for major birth defects in Malaysian births from 22 weeks gestation till one week of life, delivered at the Kinta district, Perak, Malaysia over a 14-month period using a case-controlled population-based birth defect register. Results: There were 253 babies with major birth defects in 17720 births, giving birth prevalence of 1 in 70 (1.43%) (95% CI: 0.87-1.99). The exact syndromic diagnosis of the babies with multiple birth defects could be identified in 62 (77.5%) babies. Isolated major birth defects were cardiovascular (13.8%), cleft lip and palate (11.9%), clubfeet (9.1%) and central nervous system abnormalities (7.9%). The babies with major birth defects were lighter, more premature, had higher Caesarean section rates, required prolonged hospitalization and more specialist care and had a perinatal mortality rate of 25.2%. Mothers with affected babies were older, had birth defects themselves or in their relatives, had a consanguinity rate of 2.4%, and had higher rates of previous abortions. Risk factors identified for birth defects using multivariate logistic regression were maternal insulin-dependent diabetes, previous abortions, maternal recall of exposure to teratogens during pregnancy but not lack of periconceptional folate supplementation. Conclusions: Further investigations are required to investigate the role of periconceptional folate supplementation and to confirm the above findings. Pre-natal screening for insulin dependent diabetes, counselling and investigating causes for previous abortions and public education on avoidance of teratogens may reduce birth defects in this population. A Birth Defect Register must be set up to monitor these developments in Malaysia.

Fetal Therapy: Thoracoamniotic Shunt for Macrocystic Congenital Cystic Adenomatoid Malformation of the Lung (CCAM). *R.D. Wilson^{1,2}, M.P. Johnson^{1,2}, A.W. Flake^{1,2}, H.L. Hedrick^{1,2}, T.M. Crombleholme¹, M.W. Bebbington^{1,2}, N.S. Adzick^{1,2}.* 1) Ctr Fetal Diag/Treatment/Wood, Children's Hosp Philadelphia, Philadelphia, PA; 2) University of Pennsylvania Philadelphia PA.

Objective: To retrospectively review prenatally diagnosed fetuses with macrocystic CCAM that underwent thoracoamniotic shunting(TAS) in-utero therapy and determine the pregnancy and neonatal outcome . Methods: Retrospective chart review(1998-2003) from a single treatment center was undertaken for fetuses with prenatally diagnosed macrocystic CCAM that underwent TAS. Prenatal,delivery,postnatal factors and perinatal mortality were evaluated. Results: Total 23 cases were identified. Lung right 11,left 12; male 17,female 6; hydrops 18; polyhydramnios 11; both 9; mean gestational age(weeks) at diagnosis 22.3, shunt 24.1, delivery 36.3 with mean interval shunt to delivery of 11.8 weeks. Pre and post shunt mean CCAM volume was 70.8cc and 22.7 cc respectively. Mean CCAM volume/head circumference ratio pre and post was 2.4 and 0.7 respectively (69% reduction).Delivery was vaginal 14 , primary C/S (EXIT) 8(3) ,and repeat C/S 1. EXIT delivery with fetal thoracotomy was used in 3 cases due to risk of severe respiratory compromise at birth. Perinatal outcome was live born 22, intrauterine death 1, and neonatal death 5 . Survival was 17/23 (74%)with perinatal loss 6/23 (26%).Shunt to delivery interval for survivors was 77 days compared with 20 days for neonatal death outcome .There was no gender factor for perinatal loss(Fischer's ExactTest 2-tail 0.14)as all 6 deaths were male.All macrocystic CCAM required resection within 72 hours ,majority within 48 hrs due to air trapping and pneumothorax. Conclusion: CCAM size or laterality was not predictive of outcome . Perinatal mortality was 26% in this cohort of shunted macrocystic CCAM . Delivery of the fetus in a tertiary center with MFM/OB, neonatal and pediatric surgery MD's is necessary due to the respiratory risk factors and urgent surgery required after birth.Thoracoamniotic shunt placement should be considered as a treatment option for fetuses with macrocystic CCAM and hydrops+/- polyhydramnios .

Stepwise implementation of enhanced prenatal screening in Southwestern Ontario. *C. Campagnolo¹, S. Tokmakejian^{1,2}, R. Gagnon^{1,2}, J-A. Kane¹, K. Corley¹, D. Munavish¹, V.M. Siu^{1,2}.* 1) Medical Genetics Program of Southwestern Ontario, London Health Sciences Centre; 2) University of Western Ontario, London, ON, Canada.

Triple marker maternal serum screening for Down syndrome was introduced in Ontario in 1993. With new developments, there has been an impetus to incorporate improved screening practices into the existing program. Southwestern Ontario has a population of 1.7 million, with 40% of its population living in rural areas where access to specialized ultrasound is limited. Any improvement in prenatal screening should address the needs of the entire catchment area. In April 2003, dimeric inhibin-A (DIA) was readily added to the existing triple screen (AFP, uE3, hCG), using a 1:260 risk cut-off at term. In December 2003, after intensive community and physician education, serum-only 5-marker screening was initiated, combining first trimester pregnancy associated plasma protein-A (PAPP-A) with second trimester quadruple screen, using a 1:200 risk cut-off. As certified nuchal translucency (NT) measurements became available, more women have had access to full IPS (PAPP-A, NT, AFP, uE3, hCG). With enhanced screening, a change in the magnitude of risk reported for Down syndrome was noted - positive screens tend to have higher risks and negative screens have lower risks than with the triple screen. The reported risks have been helpful in decision-making about invasive testing. The number of women choosing to have prenatal screening for Down syndrome has increased by 22% over the past year. We hypothesize that this is due to greater acceptance and increased awareness of enhanced screening. The Medical Genetics Program of Southwestern Ontario has successfully initiated an enhanced prenatal screening program for Down syndrome through stepwise implementation, providing accessibility to improved screening for all pregnant women in its catchment area within a short time period.

Eugenics revisited: testing the limits of prenatal diagnosis. *R.R. Lebel, G.F. Guzauskas.* Greenwood Genetic Ctr, Greenwood, SC.

The roots of eugenics run deep in the culture of science and society, the word having been coined by Charles Darwin's cousin Francis Galton a century ago. As with philosophy, art, education, religion, politics, and science itself, its positive pursuit is some practical improvement of humankind. When ideologic agenda are superimposed on such pursuits, the outcomes are highly problematic. Indeed, the word eugenics is frequently treated like an obscenity. Warnings about the eugenic implications of prenatal diagnostics have been on hand since the techniques were new, exemplified by such heroes of our field as Jerome Lejeune in his Allan Award address (1969). However, Charles Epstein, a contemporary leader in genetics, could without blushing employ this vocabulary word in his Allan Award address (2001). Direct to consumer marketing of genetic tests has further complicated this dialogue by legitimizing availability of tests for conditions with moderate or indeterminate pathologic implications. This exacerbates the real or perceived "commodification" of children, violating many of our ethical commitments. Consequently, genetic counseling becomes more challenging as we encounter requests for testing (implying consequent selective pregnancy termination) aimed at traits that professional geneticists doubt are appropriate justifications for such actions. A well known example of this is sex selection on social grounds; a new example is testing for late-onset health problems that are amenable to medical amelioration and management. This problem has been noticed in the popular press, as talk of designer babies gains currency while geneticists had been accustomed to treating the idea as fanciful or hyperbolic. Any effort of genetics professionals to control such a trend appears likely futile. Perceived competition between public (consumer) goals for acceptable or desirable babies, and the professional (geneticists) desire for ethical integrity, can be a source of tension in the clinical setting. We will present suggested pathways toward a more optimistic response, one that reduces that tension.

A prenatal analysis of spinal muscular atrophy in 312 Italian families: evidence for a SMA 0 phenotype? A.

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BACKGROUND: Spinal muscular atrophy (SMA) is a recessive neurodegenerative disorder, characterized by the loss of motor neurons in the spinal cord and death of motor neuron cells. SMA occurs with a frequency of 1 in 10,000 live births with a carrier frequency of 1 in 50 and is the leading genetic cause of infant mortality. Based on age of onset and severity of the disease, SMA patients are classified as type I, II, or III. All three forms of SMA are caused by loss or mutation of the telomeric survival motor neuron gene (SMN1) which is deleted in almost 95% of SMA patients. Prenatal diagnosis of spinal muscular atrophy is usually performed in high risk couples by detection of a homozygous deletion in the survival motor neurone gene (SMN1) and linkage analysis with microsatellites markers in the 5q13 region. **OBJECTIVES:** In our study, we determined the frequency of transmission of the disease-allele using data from our ten years experience in prenatal molecular studies of 312 Italian families. **METHODS:** foetal DNA was extracted by chorionic villus samples from high risk SMA pregnancies. Homozygous deletion of SMN1 exons 7 and 8 were detected by PCR and restriction analysis with DraI and DdeI enzymes as described (van der Steege et al., 1995). Linkage analysis with a set of microsatellites markers across the SMN region was performed in all families to confirm the direct results and to exclude maternal cells contamination. **RESULTS:** of 312 fetuses analysed, 118 were homozygous for the wild type allele (37,8%), 131 were carriers (42%) and the remaining 63 resulted homozygous for the mutated allele (20,2%). Chi square analysis demonstrated that frequency of transmission of the mutated allele is statistically deviant from the 50% expected in autosomal recessive disorders ($p=0.0026$). **CONCLUSIONS:** This study indicate a preferential transmission of the wild type SMN1 alleles in SMA pregnancy. We speculate that this observation is the consequence of an excess of embryonic lethality of SMN1 $-/-$. If this effect is a consequence of a complete absence of SMN products remain to be defined.

Fetal muscle biopsy for diagnosis of nemaline myopathy - a case report. *S.B. Kasperski¹, A.M. Brennan², J.E. Corteville², R.S. Finkel¹, J. Golden¹, M.P. Johnson¹, R.D. Wilson¹.* 1) Children's Hospital of Philadelphia, PA; 2) Metro Health Medical Center, Cleveland, OH.

Introduction: Nemaline myopathy is a rare, genetically and phenotypically heterogeneous congenital myopathy characterized by varying degrees of hypotonia and muscle weakness. Its diagnostic hallmark is the presence of rod-shaped "nemaline bodies" in the sarcoplasm of skeletal muscle. Five genes have been identified, all of which encode protein components of the muscle thin filament.

Fetal muscle biopsy can be informative in delineating the status of a fetus at risk for a congenital myopathy or dystrophy when less invasive diagnostic testing is negative or non-specific. We report the first known muscle biopsy characteristic of nemaline myopathy.

Case: A 43 year-old G3P2 female of Syrian descent was referred for fetal muscle biopsy for a history of nemaline myopathy. Specifically, the consanguineous couple's daughter was diagnosed with nemaline myopathy at age 3 by muscle biopsy. Her clinical course is characterized by infantile hypotonia, weakness and developmental motor delay. Molecular testing was undertaken and was uninformative. CVS performed in the first trimester of pregnancy revealed a normal karyotype.

Results: Fetal gluteal muscle biopsy was undertaken under ultrasound guidance at 22 weeks gestation. There were no apparent complications. Results were compatible with nemaline myopathy based on both histology and ultrastructural examination. With this information, the patient elected to terminate the pregnancy at 23 weeks gestation.

Conclusion: Pathologic changes characteristic of nemaline myopathy can be identified in second trimester fetal skeletal muscle tissue.

Higher contribution of gamma globin to the pool of circulating fetal RNA following intervention in complicated pregnancies. *M.L. Tjoa¹, K.L. Johnson¹, J. Jani², L. Lewi², J. Deprest², D.W. Bianchi¹*. 1) Dept. of Pediatrics, Division of Genetics, Tufts-NEMC, Boston, MA; 2) Dept. of Obstetrics and Gynecology, University Hospital Gasthuisberg, Leuven, Belgium.

Background: Fetal cell-free (CFF) mRNA in maternal blood can be isolated, amplified, and used for clinical applications. It is unknown whether the placenta or the fetal circulation is the main tissue source of CFF-mRNA. In this study, we assessed the levels of placenta-derived and fetal blood-derived CFF-mRNA in pregnant women undergoing treatment for twin-twin transfusion (TTTS) and polyhydramnios.

Methods: Plasma samples were collected from 6 non-pregnant female controls and 7 pregnant women before treatment, 30 minutes, one hour, 24 and 48 hours following obstetrical intervention. Cell-free mRNA was isolated and used for quantitative RT-PCR to determine levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), gamma globin and human placental lactogen (hPL) as markers for total cell-free mRNA, fetal hemoglobin and placental mRNA, respectively.

Results: Median levels of gamma globin mRNA were significantly higher than the median levels of hPL mRNA in all patients at all time points (Wilcoxon signed rank test, $p < 0.05$). In general, median GAPDH, gamma globin and hPL mRNA levels remained stable pre- and post-procedures. However, in one woman being treated by bipolar cord procoagulation for TTTS, all 3 mRNA levels increased post-procedure. No hPL was detected in control women.

Discussion: This study shows that fetal blood contributes more significantly to the pool of CFF-mRNA than the placenta in some clinical settings. Moreover, interventional treatment does not appear to affect levels of CFF-mRNA, which is in contrast to our previous study demonstrating elevated levels of CFF-DNA following laser ablation treatment of TTTS (AJOG 2004, in press).

Placental Volume Measured by 3D Sonography Does Not Predict Levels of Maternal Plasma Cell-Free Fetal DNA during the First Trimester. *T. Wataganara*¹, *M. Metzenbauer*², *I. Peter*³, *K.L. Johnson*⁴, *D.W. Bianchi*⁴. 1) Div of Maternal-Fetal Med, Dept of Ob/Gyn, Siriraj Hospital, Mahidol University, Bangkok; 2) Ludwig Boltzmann Inst for Clinical Ob/Gyn, Danube Hospital, Vienna; 3) Inst of Clinical Research and Health Policy Studies, Tufts-New Engl Med Ctr, Boston, MA; 4) Div of Genetics, Dept of Pediatrics, Tufts-New Engl Med Ctr, Boston, MA.

Background: Measurement of cell-free fetal (cff) DNA in maternal plasma may have clinical applications in prenatal diagnosis of Down syndrome and pre-eclampsia. Little is currently known regarding the tissue of origin of these fetal sequences. We tested the hypothesis that if the placenta is the major contributor of circulating cff DNA, then increased placental volume should be associated with higher maternal plasma cff DNA levels. **Material and Methods:** We enrolled 143 pregnant women who underwent first trimester placental volume measurements using three-dimensional ultrasonography. Maternal peripheral blood samples were drawn on the day of the scan. Cff DNA in maternal plasma was quantified by real-time PCR amplification of a Y chromosome sequence. The association between the measured placental volume and maternal plasma cff DNA level was analyzed along with relevant clinical variables. **Results:** The median (25th, 75th percentiles) maternal plasma cff DNA level was 16.9 genome equivalents/mL (10.8, 28.7). We found an association between the DNA levels, gestational age, and body mass index (BMI) of the subjects; therefore, adjustment for these factors was performed prior to further analysis. The median placental volume, as measured by 3D sonography, was 53.2 mL (43.0, 64.7), and the median placental quotient (i.e. the ratio between placental volume in mL and fetal crown rump length in mm) was 1 mm^2 (0.8, 1.1). Based on linear regression analyses, neither of the above placental measurements showed a significant association with plasma cff DNA levels. **Conclusions:** Our results did not show a significant association between either placental volume or placental quotient and maternal plasma cff DNA levels. We speculate that the extent of apoptosis within the placenta primarily affects the amount of cff DNA released into the maternal circulation.

Complete characterisation of a marker chromosome by chromosome microdissection, reverse and forward chromosome painting. *F. Mahjoubi*^{1, 4}, *A. Daniel*², *G. Peters*², *P. Malafiei*², *R. Hill*⁴, *A. Turner*³. 1) Dept Clinical Genetics, NIGEB, Tehran, Iran; 2) Cytogenetics Dept., Western Sydney Genetics Program, The New Children Hospital Westmead, NSW, Australia; 3) Medical Genetics Dept., Sydney Children Hospital, Randwick, NSW; 4) Division of Molecular Science, CSIRO, North Ryde, NSW 2113 Australia.

A small, mosaic, C-band negative marker chromosome was detected on culture of amniocytes during prenatal diagnosis related to advanced maternal age. Following spontaneous premature labour at 29 weeks gestation, a dysmorphic infant was delivered, with flat nasal bridge, short palpebral fissures, micrognathia, high forehead, low-set ears, telecanthus and corneal dystrophy. Additional folds of skin were present behind the neck, and feet and fingers and toes were abnormally long. The child died at age five days, after two days in renal failure. The origin of the marker chromosome was subsequently identified from a cord blood sample, via chromosome microdissection. Through reverse FISH, we found the marker to be an inverted duplication of the region 15q26.1-qter. FISH with alphoid satellite probe was negative, while whole chromosome 15 paint was positive. Both ends of the marker were positive for the telomeric TTAGGG probe. These data, plus the G-banding pattern, identified this as an alphoid, inverted duplicated marker, lacking any conventional centromere. We discuss the aetiology and clinical effects of this marker, comparing it to the few other reported cases of tetrasomy 15q syndrome. We also discuss the possible mechanisms that are likely responsible for this neocentromere formation.

Rapid clearance of mRNA for PLAC1 gene in maternal blood after delivery. *P. Carinci, M. Concu, I. Banzola, M. Marini, E. Caramelli, F. Pezzetti, A. Farina.* Dept of Histology and Medical Embryology, University of Bologna, BOLOGNA, Italy.

The objective of this paper was to evaluate (1) whether the presence of mRNA for the specific trophoblast gene PLAC1 in maternal whole blood is pregnancy-specific, and (2) whether delivery would result in the clearance of mRNA from maternal blood. Sixteen pregnant women at term (41 completed weeks gestation) were enrolled in the study. Blood samples were obtained before the onset of labor and 24 hr after delivery. Eight healthy donors (3 males and 5 non-pregnant women) were used as controls. Total RNA was extracted by means of ABI PRISM 6100. A quantitative evaluation was obtained by means of real-time PCR. Wilcoxon test was used to evaluate differences between time intervals. Median concentrations of PLAC1 mRNA relative to the standardization curve were 44 (2.9-675) ng/mL, and 0.48 (0.05-10.7) ng/mL respectively for pre- and after- delivery samples. Male and non-pregnant female controls did not show any signal of cDNA amplification. In conclusion, mRNA transcripts from a placenta-expressed specific gene is detectable in maternal blood and rapidly disappears after delivery. Such an mRNA provides a gender-independent marker for non-invasive prenatal gene expression profiling, and can open new prospective to monitor those conditions associated to trophoblast damage as well as preeclampsia.

High-throughput gender typing using SNP genotyping technology. *T. Bettecken¹, P. Lichtner¹, T. Strom¹, S.K. Shernan², A.A. Fox², C.D. Collard³, T. Meitinger¹, S.C. Body².* 1) Human Genetik, GSF Forschungszentrum, Neuherberg, Germany; 2) Brigham and Women's Hospital, Boston, MA; 3) Texas Heart Institute, Houston, TX.

Molecular genetic typing of gender in mammals and humans has been a longstanding issue. It has its importance by numbers in animal breeding. In humans, it is widely employed in prenatal diagnosis, especially for X chromosomal recessive conditions, but also for quality control of tested materials and forensic identification. Established methods for DNA based gender typing largely rely on FISH signals of the Y and X chromosomes and on the selective amplification and visualization of either Y chromosomal loci or XY homologous regions differing in size of PCR products. With the advent of high-throughput SNP genotyping, an opportunity exists for gender identification of large numbers of samples. The assignment of a genotypic gender to a sample in the very end of the entire genotyping process allows genotypic matching to the phenotypic gender of the proband, thus considerably reducing the probability of sample mix-up, although not completely excluding them. The female/male respectively XX/XY distribution pattern on the microtiter plates entering the high throughput SNP genotyping process is a characteristic feature of the plate batches and allows unequivocal plate batch identification and potential, although not complete, recognition of sample mix-up. We present selected markers from genes on the X chromosome and their respective pseudogenes on the Y chromosome which can serve for DNA based gender identification using high-throughput MALDI-TOF, and other technology, genotyping, together with validation data from more 1000 individuals. Five non-homogenous single base pair markers were identified within the AMELX/AMELY and GYG2/GYG2P gene pairs that had sufficient sequence similarity to allow MALDI-TOF typing. Call rates for markers were between 88.6% and 99.5% (median 94.8%) with complete genetic agreement for all assigned genotypic genders. Two phenotypic genders did not match genotypic gender assignment. Both samples were processed back-to-back and were presumably inadvertently mislabelled.

Distribution of IVS8-PolyT genotypes in an archived Cystic Fibrosis patient population affirms separate testing of reflex alleles. *A.G. Hadd, I. Wolf, J. Brown, C. Novak, C.R. WalkerPeach.* Ambion Diagnostics, Austin, TX.

PURPOSE: The purpose of this study was to associate the distribution of 5/7/9T alleles with 25 common mutations in the CFTR gene using archived DNA from a CF patient population. **METHODS:** A set of 350 affected and unaffected CF patient samples from a Minnesota clinic, archived between 1986-1994, were analyzed for 25 primary CF mutations plus PolyT reflex alleles. Amplicons for primary mutations and reflex alleles were generated in a single-tube multiplex PCR and analyzed by bead-array hybridization with allele-specific capture probes. Two separate arrays, a 52-bead array for primary mutations and a 7-bead array for PolyT reflex alleles, were used to analyze unpurified, biotinylated amplicons. Four exogenous internal fluorescence control beads were included to facilitate software background and variance calculations. Following hybridization and addition of a reporter fluorophore, normal, mutant (disease indicating), and polymorphic alleles were detected using a Luminex platform. Results from the two arrays were automatically processed and linked through database management software. Unique genotypes were confirmed by DNA sequencing. **RESULTS:** The detection of primary and reflex panel alleles within a CF patient population was demonstrated using direct hybridization to separate bead-arrays. In the 700 chromosomes analyzed, a prevalence of 5.5% for 5T and 35.5% for 9T alleles was observed. The 16 samples that were 5T/7T heterozygous were wild-type normal for the primary 25 CFTR mutations tested. Conversely, two R117H heterozygous samples were 7T homozygous. Of the samples that were deltaF508 heterozygous, 99% (71/72) were detected with a 9T allele. For deltaF508 homozygous samples, 38 of 40 were homozygous 9T. **CONCLUSIONS:** The distribution of 5/7/9T alleles was associated with primary mutations detected in a CF patient population. Within this CF patient population, deltaF508 alleles were predominately associated with 9T, and an expected prevalence of 5T alleles was observed. That none of the 5T alleles were associated with a severe CF mutation affirmed the separate reflex testing recommendations for PolyT.

High frequency of carriers for maple syrup urine disease in the Ashkenazi Jewish population. *R. Kornreich, L. Edelmann, G.A. Diaz, R.J. Desnick.* Dept Human Genetics, Mount Sinai School Medicine, New York, NY.

Maple Syrup Urine Disease (MSUD) is an autosomal recessive inborn error of metabolism caused by the deficient activity of the mitochondrial multienzyme enzyme complex, branched-chained--ketoacid acid dehydrogenase (BCKD). The BCKD complex includes four proteins encoded by the E1, E1, E2 and E3 genes. As E3 is also a component of the pyruvate dehydrogenase and -ketoglutarate dehydrogenase complexes, mutations in this subunit result in a distinct phenotype that includes lactic acidosis and branched-chain amino acidemia. Mutations in the E1 or E2 genes can cause classic, intermediate, or intermittent MSUD. Newborn screening for MSUD is currently available in 31 states, but despite early diagnosis and dietary therapy, episodes of potentially life-threatening decompensation occur with metabolic stress or infection. Although MSUD is a pan-ethnic disorder, it is more frequent in the Ashkenazi Jewish (AJ) population. In previous studies of MSUD mutations in seven AJ patients in our clinic, three E1 gene mutations (R183P, G278S and E422X), and one E2 gene mutation (E2delAT) were detected, accounting for all mutant alleles. Until now, prenatal carrier screening for this disease in the AJ population has not been routinely performed despite its increased incidence. Therefore, the carrier frequency of each of these mutations was determined by allele-specific oligonucleotide hybridization in over 1,100 anonymous AJ individuals. The combined carrier frequency for the three E1 gene mutations in this population was 1/81. Of note, only a single carrier of the E2delAT mutation (1/1125) was identified. These pilot studies suggest that prenatal carrier testing for the E1 mutations R183P, G278S and E422X will detect >99% of MSUD E1 gene mutation carriers in the AJ population, providing a prenatal carrier screening assay with high detectability.

Adaptation of a Cystic Fibrosis mutation panel to incorporate population-specific alleles. *C.R. Novak, A. Hadd, J.T. Brown, C. WalkerPeach.* Ambion Diagnostics, Austin, TX.

PURPOSE: To adjust quickly to changing genetic testing recommendations and incorporate newly revealed population-specific CF indicating alleles, a bead-array testing platform was examined. Within the CFTR gene, densely positioned mutations provide unique challenges to rapid CF testing strategies. The purpose of our study was to expand a current CF carrier-screening assay for the inclusion of European population-specific alleles (3905insT, Y1092X, E60X, S549N, 2183delAA>G, Q493X, 3199del6) without detriment to the original assay. **METHODS:** A single-tube multiplex PCR for CFTR alleles was expanded from 19 to 24 amplicons. The PCR was optimized for 10-500ng of genomic DNA purified from whole blood. Detection of added mutations was accomplished by including additional allele-specific capture probes conjugated individually to fluorescently addressed microspheres. Multiplexed PCR products were directly hybridized to the bead-array (without secondary amplification or purification). Samples were analyzed for 32 mutations in 15 seconds per patient with results automatically processed for allele ratio and genotype. **RESULTS:** The expandability and resolving power of the bead-array screening approach is best demonstrated with the mutations of Exon 11. Nine capture probes were designed to resolve 4 normal and 5 mutant alleles within a single 126-bp amplicon. Despite constraints of overlapping probe sequences, successful discrimination of S549N was implemented while maintaining fluorescence values greater than 10 times that of the variance of the background. Detection of the preexisting alleles G542X, G551D, G553X, and R560T was unaffected. The assay remained at 50 minutes of hands-on time (18% of total workflow) despite the addition of 7 population-specific alleles. **CONCLUSIONS:** Using multiplex PCR, bead-array hybridization and Luminex detection, a set of 25 CF mutations was expanded to 32 with successful detection. Data generated supports the expandability of genetic screening assays designed on the Luminex platform. This enables screening assays to be modified quickly for compliance with changing policies and modified recommendations of population-specific alleles.

Canavan pregnancy following use of a gamete donor. A case report. *D.B. Rogers¹, M. Kohan¹, S. Bhatt¹, H. Danzer², L.D. Platt³.* 1) Genzyme Genetics, Orange, CA; 2) Southern California Reproductive Center, Beverly Hills, CA; 3) Center for Fetal Medicine and Woman's Ultrasound, David Geffen School of Medicine at UCLA, Los Angeles, CA.

The growing use of assisted reproductive technologies (ART) and preimplantation genetic diagnosis (PGD) have broadened the field of specialists who must be cognizant of screening for genetic disease. Our case demonstrates the need to apply the principles of genetic screening to gamete donors based on family history and ethnicity. A 49-year-old Ashkenazi Jewish (AJ) woman and her 48-year-old AJ spouse conceived via ovum donation, and IVF with ICSI. PGD to detect chromosomal aneuploidy was performed on viable embryos prior to the successful implantation of a single embryo. An abnormal Expanded AFP screen brought the couple in for genetic counseling and second trimester ultrasonography. At that time genetic screening of the father found him to carry a mutation for Canavan disease. Although the AJ egg donor was screened negative for cystic fibrosis she had not been screened for other diseases based on her ethnicity. Amniocentesis revealed a chromosomally normal female with normal AFP. However, the fetus was found to carry two copies of the E285A mutation for Canavan disease. Canavan testing was recommended on the egg donor, but she was unavailable. Biochemical testing of the amniotic fluid confirmed that the fetus was affected with Canavan disease. Molecular analysis on fetal DNA ruled out uniparental disomy as a cause of Canavan disease in the fetus. The patient elected to terminate the pregnancy. The growth of ART through fertility specialists and the increased association with programs that offer gamete donation have necessitated a greater awareness of recommendations for genetic screening based on ethnicity and family history. In 1998 both the ACMG and ACOG recommended that persons of AJ descent be offered carrier testing for Canavan disease preferably before pregnancy. This tragic case demonstrates the essential requirement of screening prospective gamete donors before they become donors.

Neonatal detection of the 35delG mutation of the GJB2 gene in Brazil. *E.L. Sartorato¹, C.A. Oliveira¹, A.T. Maciel-Guerra².* 1) Lab de Genética Humana/CBMEG - UNICAMP, Campinas - SP, Brasil; 2) Depto. Genética Médica/FCM - UNICAMP, Campinas - SP, Brasil.

Mutations in GJB2 gene represent a major cause of congenital deafness, and a single mutation 35delG accounts for the majority of nonsyndromic hearing loss in different population. The prevalence of heterozygous 35delG carriers among hearing population is high (2-4%) in several countries where this mutation analysis was performed. Universal newborn screening for hearing loss has been widely adopted since it is known that early identification and management of deafness are important for language development and social skills. Genetic testing for hearing loss is becoming an emerging public health issue although deafness may be also due to inherited or environmental factors,. We performed genetic testing for 35delG mutation on paper dried blood spots of newborn infants from different regions of Brazil, which is the largest country in South America. The country is a melting pot of different ethnic groups including European, African slaves, and autochthonous Amerindians. Our results indicate that the frequency of 35delG carriers depend on the predominant immigration in special regions of the country. The knowledge of 35delG frequency variation must be useful for genetic counseling and may propitiate an early intervention for a substantial percentage of deaf infants.

Newborn screening for cystic fibrosis: clinical evolution of children screened at birth with an atypical *CFTR* genotype. V. Scotet¹, M. Roussey², G. Rault², V. Storni², M. Dagorne², H. Journal², P. Vigneron², M. De Braekeleer¹, M.P. Audrezet¹, C. Ferec¹. 1) INSERM U 613, Brest, France; 2) Réseau Mucoviscidose Bretagne et Pays-de-Loire, France.

Newborn screening for cystic fibrosis (CF) is implemented in whole France since 2002. A pilot experience was already set up in a western area of this country in 1989 (Brittany). The screening protocol performed in this region combined the immunoreactive trypsin (IRT) assay with the systematic analysis of three exons of the *CFTR* gene, which was followed by an extensive molecular analysis when necessary. This strategy led to the detection of new mutations and some children with an atypical genotype were identified (i.e. genotypes including abnormality not previously described or genotypes whose clinical evolution remained difficult to predict). The aim of this study was to describe the clinical outcome of the children screened with an atypical *CFTR* genotype. Until December 31st 2003, 528,310 newborns were screened for CF in Brittany. The IRT/DNA protocol enabled the detection of 190 children with an elevated IRT and two *CFTR* mutations. The diagnosis of CF was unambiguous in 172 of these newborns (90.5%). The 18 remaining children presented an atypical genotype: F508del/R117H (n=6), I507del/R117H, G551D/R117H, G551D/R553G, W846X/R117C, F508del/G149R, F508del/P205S, F508del/R347L, F508del/S977F, F508del/R1070W, F508del/D1152H, N1303K/F311L and 1078delT/F311L. At this time, 15 of these children (83%) do not present any pulmonary or digestive signs (follow-up periods: 1.5-15 y.). Six of them are more than 10-year old and 4 have not evolved towards a phenotype of CF (genotypes F508del/R347L, F508del/R1070W, F508del/D1152H, G551D/R553G). None of the 8 children carrying the R117H mutation (all associated with 7T variant) has developed a symptomatology of CF (ages: 2.0-8.9 y.). On the opposite, this clinical follow-up has enabled to confirm the deleterious effect of the identified abnormality in 3 children (G149R and F311L). This longitudinal follow-up is crucial to determine the outcome of the CF children with an atypical genotype. It has enabled to associate some abnormalities with a genotype of CF.

The prevalence of antiphospholipid antibodies in 261 spanish women with recurrent spontaneous abortion. A case-control study. *J. Carbone¹, M. Orera², E. Sarmiento¹, C. Lostau², D. Micheloud¹, M. Rodriguez-Mahou¹, E. Fernandez-Cruz¹.* 1) Clinical Immunology Unit, Hospital Gregorio Maranon, C. Dr. Esquerdo N. 46, 28007 Madrid, Spain; 2) Genetic Unit, Hospital Gregorio Maranon Madrid, Spain.

Approximately 10% of all human pregnancies end in spontaneous abortions. In the majority of such cases the etiology remains unknown, but anticardiolipin antibodies (ACA) are gaining recognition as potential causes of recurrent miscarriage. According to a case and control study we determined the prevalence of moderate-high ACA in 261 women with recurrent abortions (two or more) without apparent cause after study and compared with the prevalence in 30 women whose pregnancies were successful and with no history of abortion. IgG and IgM ACA were measured by enzymelinked immunosorbent assay on microtitre plates. Plates were coated with highly purified cardiolipin and saturated with human b2-GP-I which provide results independent of endogeneous b2-GP-I. The results for IgG and IgM ACA were reported as moderate [IgG 20-80 (GPL units) and IgM 20-80 (MPL units)] or high [IgG > 80 (GPL units) and IgM > 60 (MPL units)]. We found that 47 (18.8%) of the cases with recurrent abortions showed moderate-high ACA-IgG versus 1 (3.3%) of the control group (2-sided Fishers exact test, $p = 0.038$) with an odds ratio (OR) of 6.36. Low titer IgM-ACA were observed in 2 (6.6%) controls. We found a relation with IgG-ACA ($p = 0.011$) but not with IgM-ACA ($p = 0.32$). We concluded that moderate-high IgG-ACA are associated with recurrent abortions and increases the risk approximately 6-fold. Moderate-high IgG ACA are more specific for fetal wastage than IgM-ACA.

Integrated Prenatal Screening in Eastern Ontario: Lessons From the First 18 Months. *J.Y. Al-Aama¹, N. Lepage², J. McGowan-Jordan¹, V. Hastings¹, C. Meier³, G.E. Graham¹*. 1) Department of Genetics, Children's Hospital of Eastern Ontario and University of Ottawa, Ontario, Canada; 2) Department of Laboratory Medicine, Children's Hospital of Eastern Ontario and University of Ottawa, Ontario, Canada; 3) Ontario MSS Database and North York General Hospital, Toronto, Ontario, Canada.

In July 2002 Integrated Prenatal Screening (IPS) was implemented in the Ottawa-Gatineau region. Herein we report factors influencing our choice of IPS over FTS and outcome data for the first 18 months of the IPS program. We retrospectively compared IPS outcomes to those we would have obtained with FTS and previously obtained with MSS. From July 2002 to December 2003, following a T1 ultrasound in which fetal CRL (+/- BPD) and NT were measured, peripheral blood samples from 5530 pregnant women in Eastern Ontario and Western Quebec were analyzed for T1 PAPP-A and T2 msAFP, estriol and hCG levels. All sonographers participating in the program received Fetal Medicine Foundation Canada training. NT measurements were converted to MoMs based on a comparison to baseline sonographer-specific gestational age curves. The biochemical and NT data were combined with maternal demographic data to generate a T2 risk assessment according to the method of Wald et al. Free hCG was also measured in 4430 T1 samples and retrospectively combined with maternal demographics, NT and PAPP-A results for an FTS risk assessment. A risk cutoff of greater than or equal to 1 in 200 for a screen positive result for DS was used for both IPS and FTS; a risk cutoff of 1 in 385 was used for MSS. The IPS program yielded a DS initial positive rate of 5.9%, whereas the FTS program, had it been implemented, would have yielded a comparable value of 14.9%. MSS yielded an initial positive rate of 11%. Over a period of 1 year, IPS and FTS produced detection rates of 85-90%, while the figure for MSS was 72%. Only 3.4% of women screened with IPS underwent amniocentesis, compared with 6.0% of women screened with MSS. IPS provided screening for 60% more women than MSS with a 43% reduction in the number of invasive procedures and a considerable improvement in DS detection.

ProMBP and other early first trimester maternal serum markers may be combined to a highly efficient first trimester integrated screening. *M. Christiansen.* Dept Clinical Biochemistry, Statens Serum Inst, Copenhagen, Denmark.

The proform of eosinophil major basic protein (ProMBP) is synthesized in the human placenta during pregnancy. In the circulation, proMBP is known to exist in covalent complexes with other proteins, primarily pregnancy-associated plasma protein-A (PAPP-A/proMBP), and angiotensinogen (Ang/proMBP). The discriminating potential of the maternal serum concentrations of all proMBP complexes (total proMBP) in screening for Down syndrome (DS) was evaluated. The serum concentrations of total proMBP was determined by ELISA in 39 women with a Down syndrome fetus and 123 unaffected pregnancies in week 4 -14 of gestation, and distributions of gestational ageindependent concentration values (MoMs) were established. The performance of total proMBP as first trimester marker for Down syndrome was assessed through a Monte Carlo simulation procedure. The total concentration of proMBP was reduced to a median MoM of 0.70 in week 6 - 8 ($p = 0.015$) in DS pregnancies. Using a standardized age-distribution of pregnant women and published a priori risks for giving birth to a DS child, the detection rate (DR) for DS was estimated to be 41 % for a false positive rate (FPR) of 5% for proMBP and age. With supplement of nuchal translucency(NT), a DR of 76 % for a FPR of 5% could be obtained. Further combining these markers with SP1, a DR of 84 % was estimated for a 5% FPR and 71% for a FPR of 1%. The combination of proMBP and SP1 and other new early first trimester markers, e.g. inhibin A and ADAM12 may make early first trimester screening in combination with NT and beta-hCG and PAPP-A a nearly diagnostic test for Down syndrome and with performance clearly comparable to that of "Integrated Screening" where the results of serological screenin g in first and second trimesters are combined with NT to a risk result that is communicated to the pregnant woman in second trimester.

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Down syndrome detection using serum integrated screening. *S. Diment, A. Anderson-Miller, M.H. Quigg, J. Roberson.* Dept of Genetics, Henry Ford Hosp, Detroit, MI.

The Department of Medical Genetics at Henry Ford Hospital began offering Maternal Serum Integrated Screening (PAPP-A between 10-13 weeks gestation plus Quad test (AFP, estriol, hCG and inhibin-A) in the second trimester) in April 2003. Nuchal translucency measurements have not yet been incorporated into the screening program. In 14 months 800 women have completed the two-part screening test. Outcomes are pending on over half these women. Two Down syndrome cases have been identified. Both cases were detected in women less than age 35.

Patient A, age 20, had a risk for Down syndrome of 1 in 75 with Serum Integrated screening, 1 in 730 with Quad test and 1 in 2,700 with triple test. Patient B, age 30, had a risk for Down syndrome of 1 in 8 with Serum Integrated screening, 1 in 65 with Quad test and 1 in 120 with the triple test. In our screened population under age 35, the Odds of an Affected given a Positive Result (OAPR) was 1:15 with the Serum Integrated test. One third fewer women were called screen positive for Down syndrome compared to their results using the Quad test. This reduces patient anxiety, amniocentesis related costs and potential procedure-related fetal losses.

In this same group of screened women 11 (1.2%) were called screen positive for Trisomy 18. Trisomy 9 was diagnosed in the fetus of a 39 year old woman whose Trisomy 18 risk was 1 in 30 with the Serum Integrated test. Ultrasound findings included dangling choroid plexus, micrognathia, hypotelorism, absent nasal bone, multiple cardiac anomalies, abnormal skull shape, and shortened humeri and femurs.

While the number of patients screened is relatively small, Serum Integrated Screening has performed as predicted in our clinical setting. Acceptance among patients and referring physicians has been overwhelmingly positive.

An intervention trial of integrated serum testing (IST) for Down syndrome. *J. Haddow, G. Palomaki, G. Knight, E. Kloza.* Foundation for Blood Research, Scarborough, ME.

IST combines maternal serum measurements from the first (PAPP-A) and second (AFP, uE3, hCG, DIA) trimesters into a single risk calculation for interpretation in the second trimester. In this trial, IST was systematically introduced to primary care practices throughout our state. This is the first time that this more complex screening protocol has been formally assessed in the U.S. healthcare system (MCH grant MC00195). If found feasible, IST can potentially reduce the false positive rate to up to half that found with the triple test while maintaining detection. Furthermore, it sets the stage for full integrated screening, when reliable nuchal translucency measurement becomes more widely available. During 2 years of enrollment, 61% of all women screened by us in Maine 11,159 consented to IST and provided a first trimester sample. 9,723 enrollees (87%) also provided a second trimester sample; 950 of these were found to have been sampled outside of the acceptable first trimester window of 8-13 weeks (92% too early). 1,436 women did not provide a second trimester sample (40% miscarried, 31% declined further testing, 17% opted for amniocentesis. Among 8,773 women with IST interpretations, 3.2% were screen positive. This is 29% lower than the 4.5% that would have occurred if the triple test had been used (risk cut-off levels are selected to yield a 70% detection rate for each test combination). Among ultrasound-dated pregnancies, the rates were 2.7% and 4.5% (40% reduction). Follow-up to date indicates a 69% detection rate for IST, consistent with expectation. This trial shows that IST can be successfully introduced into routine practice with the following cautions: 1) IST among LMP dated pregnancies is less effective and some samples may be obtained too early; 2) additional staff time is needed to remind offices to obtain overdue samples; 3) to achieve full efficiency, ultrasound dating needs to accompany the first serum sample; 4) sample matching requires specialized software and staff involvement; 5) the PAPP-A assay requires careful monitoring and appropriate corrective actions.

Outcome analysis of pregnancies screening positive for Smith-Lemli-Opitz syndrome SLOS). *S. Langlois, S. Dougan, G. Hulait, H. Vallance, L. Armstrong, M. Van Allen.* Depts. of Medical Genetics and Pathology, Children's and Women's Health Centre, Vancouver, Canada.

SLOS is an autosomal recessive disorder characterized by growth retardation, developmental delay and minor / major anomalies. It is caused by a deficiency of 7-dehydrocholesterol reductase. Palomaki et al (2002) described a screening approach for the identification of pregnancies at high risk of having a fetus with SLOS. This study describes our experience with second trimester MSS for SLOS.

Between August 2002 and February 2004, 25,211 pregnancies had a second trimester MSS. Using a risk cut off level of 1/50, 75 MSS were positive for SLOS. 2 cases were reinterpreted due to wrong dating. Of the 73 positive screens, only 13 cases screened positive only for SLOS. The other 60 positive screens were positive for SLOS and either trisomy 18 or trisomy 21 or both. Some cases were also positive for open spina bifida. 6 cases were lost to follow up.

Prenatal investigations led to a diagnosis in 59 cases: ultrasound identified an intrauterine fetal demise in 37 pregnancies and anencephaly in 4 cases; 8 pregnancies had a chromosomal abnormality (3 trisomy 18, 1 trisomy 9, 3 triploidy and 1 case of 45,X). Steroid sulfatase deficiency (STS) was diagnosed in 10 pregnancies, four with a positive family history, 6 without. In 5 of those 6 cases, molecular analysis of amniocytes was done and a deletion of the STS gene was found in 4 cases.

Postnatally, congenital adrenal hyperplasia was diagnosed in one case. Three newborns had normal exams and neonatal courses. One of these infants is being investigated at 9 months for possible delay. Pregnancy outcome information is pending on 4 cases.

In summary, although no diagnosis of SLOS was made (FPR 0.28%), a positive MSS for SLOS is associated with a high positive predictive value for chromosomal abnormalities, intrauterine fetal demise and STS deficiency. Patients positive for SLOS on MSS require prompt referral for assessment and counselling.

Invasive Trophoblast Antigen (ITA) levels in second trimester twin pregnancies. *J.E.S. Lee¹, E. Carlton¹, P. Petersen¹, C. Maglasang²*. 1) Quest Diagnostics, San Juan Capistrano, CA; 2) Quest Diagnostics, Teterboro, NJ.

Introduction: ITA is increased in women carrying a fetus with Down syndrome. Before ITA can be incorporated into routine screening programs, the relationship between levels observed in singleton and twin pregnancies must be determined. We have compared serum ITA levels in singleton and twin pregnancies during the second trimester.

Methods: Serum samples were collected during the second trimester (14.1-22.7 weeks) from 582 women carrying one fetus and 145 women carrying twins. ITA was measured using an immunochemiluminescent assay performed on the Nichols Advantage instrument. AFP, hCG, uE3 and dimeric inhibin A (DIA) were measured using commercially available immunoassay kits. Results were converted to multiples of the median (MoM). ITA MoMs were adjusted for maternal weight. All other MoMs were adjusted for weight and race. Maternal weights ranged from 108-315 pounds and were truncated between 120-220 pounds for ITA. Women carrying twins were white (50%), black (15%), Hispanic (17%), Asian (10%) and other races (8%). The twin pregnancies were dated by ultrasound (65%) or LMP (35%).

Results: In twin pregnancies, the median ITA level was 2.19 MoM, the \log_{10} mean was 0.380, and the \log_{10} standard deviation (SD) was 0.369. In singleton pregnancies, the median, \log_{10} mean, and the \log_{10} SD were 1.00, 0.011, and 0.307, respectively. While ITA MoMs were not affected by the method of gestational dating, they were affected by race. Slightly higher MoMs (2.37) were observed in Hispanic women with twins and lower MoMs (1.26) were observed in Asians. The median MoMs of AFP, hCG, uE3 and DIA in twin pregnancies were 2.04, 1.79, 1.42 and 2.31, respectively. In singleton pregnancies, the median MoMs were all 1.00. The \log_{10} SDs in twin pregnancies were similar to those in singleton pregnancies.

Conclusions: ITA levels in twin pregnancies are approximately twice (2.19 MoM) those in singleton pregnancies in the second trimester. This relationship can be used when calculating pseudo-risks for Down syndrome in twin pregnancies.

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Review of enhanced prenatal screening performance in Ontario, Canada. *C. Meier, T. Huang, A.M. Summers, P.R. Wyatt.* Ontario MSS Database, Genetics, North York General Hospital, Toronto, ON, Canada.

Ontario is Canada's most populous province with approximately 130,000 live-births per year. Traditional second trimester, three-marker maternal serum screening (MSS) for Down syndrome, open neural tube defects and trisomy 18 has been offered as a tax funded service to the women of Ontario since 1993. The utilization of MSS has remained consistent with approximately 50% of live-birth pregnancies receiving screening. In 1999, integrated prenatal screening (IPS) which combines the results of biochemistry markers in the first and second trimester with first trimester ultrasound was introduced on a small scale in one region and rapidly expanded across the entire province. First trimester screening (FTS) was introduced in 2002 and has also become widely used. Dimeric inhibin-A (DIA) was also added to IPS and MSS tests in some areas in 2003. Currently, approximately 50% of the 6,000 monthly screening results issued in Ontario are based on some form of enhanced prenatal screening test. Enhanced prenatal screening, like MSS, is more highly utilized in urban areas due to easier access to medical care, blood collection and ultrasound facilities; its use has spread to include most rural areas as well. Between July 1999 and March 2004, approximately 55,000 women had IPS or FTS and 239,000 had MSS. The performance of IPS, FTS and four-marker MSS methodologies were assessed, with respect to false-positive rates (FPR), theoretical detection rates (DR), and utilization on a regional basis. Overall, the introduction of enhanced screening in Ontario appears to have both reduced the mean FPR and increased the mean DR.

Detection of cell free placental DNA in maternal plasma: direct evidence from three cases of confined placental mosaicism. *S. Miura¹, K. Miura¹, H. Masuzaki¹, N. Niikawa², T. Ishimaru¹.* 1) Dept of OB/GYN, Nagasaki Univ Biomed. Science, Nagasaki, Japan; 2) Dept of Human Genetics, Nagasaki Univ Biomed. Science, Nagasaki, Japan.

Objective: Cell-free fetal DNA released into maternal plasma is useful for noninvasive prenatal diagnosis, but no definitive evidence has been provided for its origin. Confined placental mosaicism (CPM) is defined by the presence of abnormal karyotypes only in the placenta. If a trisomic allele is detected in maternal plasma with CPM, this is direct evidence for placental DNA in maternal circulation. Here we report the result of a study that supports the presence of placental DNA in maternal plasma. **Material and Methods:** We analyzed parent-child transmission patterns of alleles at various dinucleotide repeat polymorphism loci in 9 cases of confined placental mosaicism (CPM). Cellular DNA was extracted from leukocytes of the neonates and their parents, and cell-free DNA was from maternal plasma (1.6 ml) collected before and after delivery, using Qiagen blood mini Kit (Qiagen, Hilden, Germany). Placental DNA was extracted after delivery. All these study protocols were approved by the Committee for the Ethical Issues on Human Genome and Gene Analysis in Nagasaki University, and written informed consent was obtained from all the women. **Results:** Diploid cells in three of the 9 neonates with CPM had one each of parental alleles, whereas trisomic cell in their placentas contained one maternal and two paternal alleles. In all of them, one paternal allele that was absent in the neonates was present both in trisomic cells of the placenta and in cell-free DNA from maternal plasma. **Conclusion:** Our results not only provide direct evidence for the presence of placental DNA in maternal plasma but also indicate possible usefulness of such DNA for noninvasive prenatal diagnosis of genetic diseases and screening for placental dysfunction.

Maternal serum invasive trophoblast antigen (ITA) in first trimester trisomy 18 pregnancies. *G. Palomaki¹, G. Knight¹, L. Neveux¹, R. Pandian², J. Haddow¹.* 1) Foundation Blood Research, Scarborough, ME; 2) Quest Diagnostics Nichols Institute, San Juan Capistrano, CA.

This initial report of maternal serum ITA in first trimester trisomy 18 pregnancies indicates that ITA performs comparably to free β -subunit of hCG (free β). Elevated levels of ITA (previously called hyperglycosylated hCG) in maternal serum and urine are known to be predictive of Down syndrome in both first and second trimesters. If ITA was to be substituted for hCG (or free β) for Down syndrome screening, it would be important to define its usefulness for identifying trisomy 18 pregnancies, as well. To answer this question, we used banked sera from a prospective observational trial (Haddow JE et al., *NEJM*, 1998;338:955). Maternal serum samples were obtained prior to CVS or early amniocentesis in 4,412 women, none of whom was having the procedure because of abnormal chemistry or ultrasound measurements. Twelve first trimester maternal serum samples from trisomy 18 pregnancies were identified; each was matched with 5 controls for gestational age (between 9 and 13 weeks), maternal age and race, recruitment center, and storage time. We assayed these never-thawed aliquots for ITA per manufacturer's instructions, using Nichols Institute Diagnostics automated platform (Advantage).

We derived day-specific reference ranges from the 60 chromosomally normal pregnancies, and all 72 ITA results were expressed as weight-adjusted multiples of the median (MoM). hCG, PAPP-A and free β were measured fresh, as part of the original study. Unbiased estimates for median ITA, hCG, free β and PAPP-A levels in trisomy 18 pregnancies are 0.18, 0.40, 0.23, and 0.29 MoM, respectively. At a 5% false positive rate, corresponding univariate trisomy 18 detection rates are 58%, 50%, 58% and 67%. Detection rates for free β and PAPP-A are consistent with published reports. Substantially equivalent screening performance for trisomy 18 can therefore be achieved by substituting ITA for either hCG or free β in first trimester Down syndrome screening.

Automated Imaging versus Manual Scoring for Rare Cell Detection: Man or Machine? *K.L. Johnson, H. Stroh, K. Khosrotehrani, D.W. Bianchi.* Division of Genetics, Department of Pediatrics, Tufts-New England Medical Center, Boston, MA.

Background. Fetal cell detection for prenatal diagnosis or study of microchimerism requires an accurate, efficient and reproducible microscopy method. Our objective was to compare manual scoring to automated scanning on the same specimens for detection of either nuclear or cytoplasmic/membrane signals.

Methods. Nuclear X and Y chromosome FISH signals were detected on slides of calibrated mixtures of male umbilical cord blood cells with $\sim 10^5$ female cells, paraffin-embedded tissue specimens from women with male fetal cell microchimerism, and post-termination blood from a woman with a 47,XY,+21 fetus. Cytoplasmic or membrane signals were detected on nucleated cells isolated from first trimester maternal blood stained with FITC-anti-trophoblast antibody HLA-G and blood smears from green fluorescent protein (GFP+) mice (expressed in $\sim 50\%$ of nucleated cells). For manual scoring (at 400X), the number of cells located and scoring time were recorded. For automated scanning (Metasystems), instrument time, manual review of gallery images, and number of cells located were recorded. Nuclear and cytoplasmic/membrane signal detection were performed at 200X and 100X magnification, respectively.

Results. For nuclear signal detection in known mixtures, the number of target nuclei located by manual and automated microscopy was highly correlated ($r=0.94$). Overall, nuclear signal detection by automated scanning required 3-fold more time than manual scoring, whereas for cytoplasmic/membrane signal detection automated scanning required 3-fold less time than manual scoring.

Conclusions. In general, the accuracy of automated and manual microscopy for rare cell detection is comparable. However, automated scanning is more rapid than manual scoring when low magnification (100X) can be used, while manual scoring is more efficient when higher magnification is necessary (200X). Due to the consistency and reproducibility of automated microscopy, it may have advantages for the localization and characterization of rare cells.

Angelman Syndrome and Beckwith-Wiedemann Syndrome methylation testing in a prospective series of 84 IVF pregnancies. *L. Ross¹, L. Black², J. Johnson³, L. Beischel³, S. Bhatt¹, J. Goldberg²*. 1) Genzyme Genetics, Orange, CA; 2) California Pacific Medical Center, San Francisco, CA; 3) Shodair Children's Hospital Genetics Laboratory, Helena, MT.

Animal cloning, molecular studies of animal embryos, patient registries, and case reports have suggested an association between assisted reproduction and an increased risk for defects in epigenesis. An apparent mechanism is loss of maternal methylation of certain imprinted genes including those causing Angelman Syndrome (AS) and Beckwith-Wiedemann Syndrome (BWS). Of published postnatal gene methylation analyses of AS and BWS patients born after IVF, 3/3 AS patients had hypomethylation of the maternal SNRPN gene, and 13/14 BWS patients had hypomethylation of the maternal copy of KCNQ1OT1 (also called LIT1). Prenatal testing is available for these two conditions and the question remains whether testing should be routinely offered to patients following IVF/ICSI procedures. Our purpose is to contribute to risk determination for methylation defects causing AS and BWS in IVF pregnancies by following a large series of prospectively ascertained patients. Patients were informed of and offered this testing during prenatal genetic counseling. In our series, all patients had undergone IVF and were also of advanced maternal age. Eighty-four pregnancies were tested for AS and 79 of these were also tested for BWS. All fetuses had normal karyotypes and no ultrasound anomalies were noted. Our series included 15 pairs of twins and 2 single fetuses from different twin pregnancies. Thirteen of the samples tested were CVS, and the remaining 71 were obtained from second-trimester amniocentesis. Forty-one of the 84 pregnancies were conceived with ICSI in addition to IVF. The DNA testing was done on cultured samples and designed to detect abnormal methylation patterns of the SNRPN and KCNQ1OT1 genes. All AS and BWS methylation test results were negative. To our knowledge this is the first set of prospectively obtained prenatal data on AS and BWS methylation status in IVF pregnancies. Future studies will expand the data set and help ascertain the true risk for methylation defects after IVF/ICSI procedures.

Development of multiplex PCR, bead-array detection and automated data analysis for complex genetic testing: the Ashkenazi disease model. *J.T. Brown, A.G. Hadd, C. Lahey, C.R. Novak, C. WalkerPeach.* Ambion Diagnostics, Austin, TX.

PURPOSE: Carrier screening for large numbers of heritable mutations is challenging and frequently achieved by performing multiple laborious techniques. The purpose of our study was to develop a rapid test procedure combining single-tube amplification, single-tube detection and automated data analysis for complex genetic testing. A panel of diseases prevalent in the Ashkenazi Jewish population served as the feasibility model. Included were allelic determinations for 8 diseases that have a combined carrier rate of 1:6, Tay-Sachs, Gaucher I, Niemann-Pick A and B, mucopolidosis IV, familial dysautonomia, Canavan, Bloom syndrome, and Fanconi anemia C. **METHODS:** We amplified 20 regions of the genome in a single multiplex PCR. Detection was a single bead-array containing 47 allele-specific capture probes conjugated individually to fluorescently addressed microspheres and 4 exogenous internal fluorescence control beads (included for software calculations of background and variance). Biotinylated amplicons were hybridized to the bead-array in standard thermal cyclers without intervening purification steps. Stringency was maintained without post-hybridization washes. After incubation with reporter fluorophore, reactions were analyzed by the Luminex platform to identify microsphere/amplicon interactions. **RESULTS:** A single assay was developed that combines multiplex PCR and bead-array hybridization to identify the normal and mutant forms of 24 alleles frequent among Ashkenazim. Analysis of 96-well plates was completed in 4.5 hours with 50 minutes (18%) of hands-on time. Fluorescence values were greater than 40 times the variance of the background, indicating a reliable, robust system. The software allowed selection of specific panel alleles for analysis and reporting on a patient-by-patient basis.

CONCLUSION: Combining multiplex PCR amplification with bead-array detection is a successful strategy for rapid genetic testing and facilitates the consolidation of multiple techniques into one assay. Further, analysis software can address ethical and policy considerations by revealing only those results which have been requested by the physician or genetic counselor.

Molecular analyses of monozygotic twins with cytogenetic discordance for trisomy 21. *J.-L. Blouin^{1,2}, S. Gagos¹, M. Gagnebin², S. Deutsch², C. Bürgi², C. Gehrig², R. Lyle², M.A. Morris^{1,2}, S.E. Antonarakis^{1,2}, S. Dahoun^{1,2}.* 1) Medical Genetics, University Hospitals of Geneva, Switzerland; 2) Genetic Medicine and Development, University School of Medicine, Geneva, Switzerland.

We report here a case of discordant monochorionic/diamniotic twin pregnancy for trisomy 21 in a 31 year old, primipara mother. This pregnancy was the result of an in vitro fertilization because of male infertility. An amniocentesis (at week 13 5/7) was performed following ultrasound signs of hydrops and hygroma colli. The karyotype (transplacental) revealed a non-mosaic trisomy 21 in twin 1 (47,XX,+21[7]) and a mosaic trisomy 21 in twin 2 (47,XX,+21[2] / 46,XX[19]). After genetic counseling a non-selective termination of pregnancy was performed. Examination of post mortem tissues (skin, kidney, lung) after an informed consent, revealed a trisomy 21 in twin 1 and a normal karyotype 46,XX in twin 2 (there was only one trisomic 21 cell in 100 cells of cord blood). Microsatellite analyses and quantification by Real-Time PCR on DNAs from 2 different tissues (hand, lung) in each twin sibling confirmed common genetic background for all loci tested, and trisomy 21 in twin 1. There was chromosome 21 allele sharing in twins 1 and 2; maternal alleles were however duplicated in the trisomic twin 1. Interestingly, twins 1 and 2 received different maternal alleles for markers of the most distal 2 Mbp of 21q. There is a complicated sequence of events that could explain these data. One scenario is that of an initial trisomy 21 zygote from a non-disjunction at meiosis II (recombinant and non-recombinant chromatids), followed by an early mitotic error that resulted in normal karyotype (containing one maternal 21), and trisomy 21 cells containing two copies of the other maternal chromosome 21. Alternative mechanisms could also explain the data. Regardless of the twinning mechanism, these samples can be used to assess transcriptome differences due to the presence of an extra chromosome 21 in an otherwise identical genetic background.

Coagulation and fibrinolysis factor gene polymorphisms in women with prior preeclampsia. *J. Girouard, S. Levesque, C. Rancourt, J.C. Forest, Y. Giguere, F. Rousseau.* CHUQ, Centre de recherche de l'hopital St-Francois d'Assise, Universite Laval, Quebec, Canada.

Preeclampsia is a pregnancy disease characterised by hypertension and proteinuria occurring after 20 weeks of gestation in women. It is an important cause of foetal and maternal morbidity/mortality and there is no current treatment other than delivery. This disorder is associated with a hypercoagulable state and can sometimes evolve to coagulation complications. We studied different gene polymorphisms of proteins involved in the coagulation and fibrinolysis pathways in order to evaluate their potential role in the pathophysiology of preeclampsia. Prothrombin (G20210A), factor V (R506Q), factor VII (R353Q and -323 10 pb ins), factor XIII (V34L), beta-fibrinogen (G-455A), platelet glycoprotein IIIa (L33P) and platelet activator inhibitor-1 (promoter 4G/5G) gene polymorphisms were studied in 307 women with prior preeclampsia matched for age at the index pregnancy and BMI with 603 controls. All women in this study were of French Canadian origin. Genotypes distribution followed Hardy-Weinberg equilibrium for all polymorphisms except for the platelet glycoprotein IIIa polymorphism among cases ($p = 0,03$). Genotype and allele frequencies were similar between women with prior preeclampsia compare to controls for all the polymorphisms studied. We also look at the severe preeclampsia subgroup ($n = 185$) and their controls but no differences were found. Combined carrier status analysis for *F5* and *F2* genes showed similar frequencies between groups (3,9 % vs 4,1 % in controls). In conclusion, our results suggest that common coagulation and fibrinolysis factor gene polymorphisms do not play an important role in the pathophysiology of preeclampsia, at least in our population.

FISH validation of preimplantation genetic diagnosis (PGD). *S.A. Berend¹, D. Crenshaw¹, S. Pritchard², M. Sapeta¹, K. Ivani³, J. Russell², F. Bischoff⁴, J. Wang⁵, A. Lamb¹, J.B. Ravnan¹.* 1) Genzyme Genetics, Santa Fe, NM; 2) Delaware Institute for Reproductive Medicine, Newark, DE; 3) Reproductive Science Center, San Ramon, CA; 4) Baylor College of Medicine, Houston, TX; 5) Genzyme Genetics, Pasadena, CA.

Preimplantation genetic diagnosis (PGD) is performed in conjunction with in vitro fertilization (IVF) and allows genetic testing of embryos prior to uterine transfer. PGD allows at-risk couples to avoid the transfer of aneuploid embryos, thus decreasing the spontaneous miscarriage rate. No guidelines exist for the validation of PGD; therefore, general guidelines by the American College of Medical Genetics (ACMG) for the validation of probes for fluorescence in situ hybridization (FISH) were used. We present approaches from two different laboratories. As it is difficult to obtain blastomeres for validation purposes, initial studies were performed on cells from chorionic villus samples (CVS), blood, and products of conception (POC). The first validation protocol involved testing the FISH probe mixture (13,15,16,17,18,21,22,X,Y) on trophoblastic cells from direct preparations of CVS. Five direct-prepared CVS slides were used to determine probe sensitivity which ranged from 86% to 100% for the probes tested. We also tested the probe set on cells from known abnormal CVS and POC specimens. All abnormalities were identified using this protocol. After gaining experience of FISH on normal and abnormal CVS and POC cases, we then tested the probes on blastomeres from abnormally-fertilized embryos (64 nuclei, 1 normal, 54 abnormal, 2 inconclusive results, 7 no results). The second validation protocol involved two probe mixtures (1st-13,18,21,X,Y and 2nd-8,9,15,16,22). Probe sensitivity was tested on blood cells (1st probe mixture on 200 nuclei, 2nd probe mixture on 100 nuclei) which ranged from 98% to 100%. Initial validation of the 1st probe set was performed on dissociated frozen embryos (357 nuclei, 255 normal, 102 abnormal), then on dissociated discarded fresh embryos (185 nuclei, 155 normal, 22 abnormal, 8 no results). Finally, single blastomeres were tested with both probe sets (114 nuclei, 30 normal, 76 abnormal, 1 no results).

Analysis of single cell diagnosis: mutations in the HEXA gene. *K. Dotan, B. Goldman, G. Barkai, J. Peri, L. Peleg.*
Human Genetics, Sheba Medical Center, Ramat Gan, Israel.

Tay Sachs disease (TSD) is a severe autosomal recessive disorder, lethal in its common infantile form. Mutations in the HEXA gene which impair hexosaminidase activity underlie the disorder. TSD is relatively frequent among the Ashkenazi Jewish population, where the carrier frequency is 1/29. Two mutations are responsible to nearly 100% of the infantile form in this population: 1278 +TATC (73% of the carriers) and IVS12 +1G to C (13%). A third mutation (G805A, 4% among carriers) causes an adult-onset form usually in compound with the common one. PGD, preimplantation genetic diagnosis was performed in a few centers following previous terminations of affected pregnancies. Our aim was to study different aspects of single cell diagnosis: amplification efficiency and allele-dropout (ADO) rates in a range of multiplexes, concurrently with analyzing the most prevailing detection techniques. The study included various cell types: lymphocytes from peripheral and cord blood, cultures of amniotic fibroblasts and chorionic cells and blastomeres). A set of nested PCR protocols were designed to detect each of the three mutations and 1st round multiplex and simultaneous PCR for compound heterozygotes. In addition we surveyed the TSD carrier population for highly polymorphic markers flanking the HEXA gene. Therefore each allele could be "mini-finger printed" by two markers (D15S1050, D15S188) via multiplex reactions with each protocol. We could detect all mutations by conventional electrophoresis and computerized systems such as ALF Express (Pharmacia) and ABI Prism 3100 Analyzer using fluorescently labeled primers. 150 single cells (from heterozygous individuals) were analyzed. Amplification rate was similar in all cell types (86.7%). However ADO rates was significantly lower (1.3%) in cultured fibroblasts than in lymphocytes (12%). We think that ADO rate is low in dividing cells whose chromatin is less compact and the DNA is more accessible. PCR failures are probably due to other factors in the reactions. We anticipate reliable amplification of the two alleles in the case of blastomeres since they are in an actively dividing stage.

Preimplantation Genetic Diagnosis in Maastricht: An overview. *J. Herbergs¹, J. Dreesen¹, E. Coonen², C. De Die-Smulders¹, J. Den Hartog², M. Drüsedau¹, J. Dumoulin², G. De Wert³, J. Engelen¹, J. Evers², J. Geraedts¹, H. Smeets¹.*
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Preimplantation Genetic Diagnosis (PGD) is an alternative for prenatal diagnosis for couples at risk of transmitting a genetic disorder to their offspring. The genetic diagnosis of the early cleavage stage embryo can prevent couples from making the difficult decision of pregnancy termination in case of an affected foetus after prenatal diagnosis or can significantly increase the chance of an ongoing pregnancy, in case of recurrent pregnancy loss. PGD in Maastricht started at the end of 1994 with FISH sex selection soon followed by PCR analysis of a specific single gene disorder. The first FISH translocation analysis was done in 1997. In almost nine years 237 PGD cycles were started in 106 patients. In the first year PGD was performed for only two different X-linked disorders. Currently PGD sex selection has been performed for 19 different XL disorders. Furthermore, 14 different chromosomal aberrations have been diagnosed as well as 10 different single gene disorders. This involved 177 ovum pick ups (OPU) and the analysis of 1236 embryos. Of the analysed embryos 507 were either not affected by the disorder investigated or of the female gender in case of a sex selection. In 147 cycles an embryo transfer (ET) could be performed resulting in, 37 singleton, 9 twin and one triplet pregnancy, of which 6 ended in an early abortion. From the triplet pregnancy one child was a stillborn. In total 40 healthy babies were born with no major complications. Nine pregnancies are still ongoing. Conclusion: In the past years the number of PGD cycles and different disorders for which PGD can be offered has steadily grown and PGD has proved to be a successful alternative for prenatal diagnosis with pregnancy rates of 27% per OPU and 32% per ET.

Preimplantation development of autosomal monosomies depending on their origin. *A. Kuliev, J. Cieslak, Z. Zlatopolsky, Y. Illkevitch, I. Kirilova, Y. Verlinsky.* Reproductive Genetics Inst, Chicago, IL.

We have previously demonstrated 3:1 disomy/nullisomy ratio in oocytes following meiosis I, which is in agreement with predominance of trisomies over monosomies in spontaneous abortions. With sole exception of monosomy 21, autosomal monosomies are not compatible with postimplantation development and have never been detected in recognized pregnancies or at birth. On the other hand, significantly higher prevalence of autosomal monosomies over trisomies has recently been reported in cleaving embryos, suggesting their possible postzygotic origin through mitotic nondisjunction or anaphase lag in the first cleavages. To investigate the developmental outcome of autosomal monosomies, detected by polar body or blastomere analysis, these embryos were followed up to the blastocyst stage, and the resulting blastocysts were re-analyzed for the chromosomal status by fluorescent in situ hybridization (FISH) analysis using commercial probes specific for five chromosomes, including chromosomes 13, 16, 18, 21 and 22. A total of 3140 nuclei were analyzed from oocytes and embryos, which were obtained from women of average age of 38.6 (± 3.6) years. A total of 134 monosomic embryos were followed up overall, 51 (38%) of which were detected by polar body testing (monosomy 13 3, monosomy 16 10, monosomy 18 5; monosomy 21 14; monosomy 22 17, and complex monosomies 2), and 83 (62%) by blastomere analysis (monosomy 13 10, monosomy 16 11, monosomy 18 10; monosomy 21 15; monosomy 22 21, and complex monosomies 16). The proportion of monosomy confirmation in the resulting blastocysts was significantly higher (88.1%) for prezygotic, compared to postzygotic monosomies, confirmed in 59.6%, the remaining showing normal karyotype or mosaicism. 28 (59.6%) of 47 of prezygotic monosomies developed to blastocyst stage, compared to 32 (46.4%) of 69 postzygotic monosomies, suggesting, that autosomal monosomies are compatible with preimplantation development, irrespective of the origin, and are probably being lost during implantation.

PCR-based aneuploidy testing for chromosomes 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 15, 16, 17, 18, 19, 21 and 22 in cleaving embryos combined with preimplantation genetic diagnosis (PGD) for Mendelian disorders. *S. Rechitsky, A. Kuliev, T. Sharapova, K. Lazyuk, S. Ozen, O. Verlinsky, I. Barsky, Y. Verlinsky.* Reproductive Genetics Inst, Chicago, IL.

It is becoming a current PGD practice to test for single gene disorders together with chromosomal abnormalities, so the same single cell is tested for both genetic and chromosomal disorders. This is done by PCR-based testing for chromosomal aneuploidies, which is based on the patterns of alleles that uniquely identify an individual, relying on a multiplex fluorescent PCR of low template DNA. We performed single cell DNA fingerprinting for detection of aneuploidies for chromosomes 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 15, 16, 17, 18, 19, 21 and 22. Overall, 2074 blastomeres were tested, the number of blastomeres tested for each chromosome ranging from a few for chromosomes 3 and 9 to over hundred for chromosomes 6, 7, 11, 13, 16, 18, 19, 21 and 22, so the individual aneuploidy prevalence was evaluated only for these chromosomes, which was 11%, 5%, 7%, 10.1%, 6.7%, 10.2%, 7%, 16.9% and 7.6%, respectively. Overall aneuploidy prevalence of 42% was evaluated based in the series of 276 blastomeres, in which copy number of chromosomes 13, 16, 18, 21 and 22 was detected in the same blastomeres. No significant monosomy/trisomy ratio differences were observed, opposite to the recently reported findings of fluorescent in situ hybridization (FISH) analysis at the cleavage stage, despite monosomy/trisomy ratio variations for individual chromosomes. Errors of two or more chromosomes were observed in one third of aneuploid blastomeres, in agreement with previous FISH data in oocytes and blastomeres. Further data collection will be required to exclude a possible monosomy overestimate due to allele drop out in single blastomere PCR, as well as trisomies underestimate due a detection failure of extra maternal or paternal chromosomes because of indistinguishable homologs sharing the same polymorphic markers. The data show that testing for copy number of chromosomes may in future allow avoiding the transfer of chromosomally abnormal embryos in PGD for single gene disorders in couples of advanced reproductive age.

Preimplantation genetic diagnosis for Myotonic Dystrophy. *P. Renbaum¹, T. Eldar Geva², G. Chicco¹, B. Brooks², E.J. Margalioth², E. Levy Lahad¹, G. Altarescu¹.* 1) Genetic Unit, Shaare Zedek Medical Center, Jerusalem, Jerusalem, Israel; 2) IVF Unit, Shaare Zedek Medical Center, Jerusalem, Israel.

Myotonic dystrophy, the most common form of inherited muscular dystrophy in adults, is an autosomal dominant disease caused by expansion of unstable CTG repeats in the 3' untranslated region of the DMPK gene. Expanded CTG repeats cannot be visualized directly by PCR, and consequently preimplantation diagnosis using PCR is based on amplification of the normal allele. This strategy alone has been shown to be susceptible to misdiagnosis in PGD. The main obstacles in single cell PCR are the high rate of allele drop out (>20% in single blastomeres) and DNA contamination. Ideally, linked STR polymorphic markers are amplified in addition to the normal DMPK allele providing for higher accuracy. We used fluorescent labeled primers for PCR followed by fragment analysis on an automated DNA sequencer for 5 STRs, and restriction enzyme analysis for additional SNP markers, for a total of 8 reactions. We report two women with myotonic dystrophy that underwent PGD. In both families the females were affected and the males healthy. We used 7 highly polymorphic markers (D19S207, D19S219, D19S595, D19S393) and SNPs in introns 9, 5, and 11, in a multiplex reaction together with the DMPK repeat allele. This analysis was performed on Polar Bodies I and II, diminishing the need to perform blastomere biopsy. One marker, D19S393, used in both families, has not been reported in PGD for myotonic dystrophy. Using only polar body biopsy, 4 out of 10 embryos were predicted to have mutation free maternal alleles in family I, and three embryos were transferred back to the patient, resulting in an ongoing singleton pregnancy. In family II only 1 out of 10 original embryos in the first cycle and 2 out of 6 embryos in the second cycle were predicted to have a mutation free maternal allele, and these embryos were transferred to the patient. These transfers did not result in pregnancy, and the family is currently undergoing an additional PGD cycle. Presented data demonstrate the usefulness of polar body analysis combined with a large number of highly polymorphic markers for PGD diagnosis.

Preimplantation genetic diagnosis (PGD) for three types of Charcot-Marie-Tooth (CMT) disease. *T. Sharapova, S. Rechitsky, Y. Verlinsky.* Reproductive Genetics Institut, Chicago, IL.

CMT represents clinically and genetically heterogeneous group of hereditary peripheral neuropathies, affecting 1 in 2,500. PGD has previously been applied in 5 couples with CMT1A, the most frequent autosomal-dominant type of CMT caused by 1.5 Mb tandem duplication on chromosome 17p11.2-12, presenting complexity of diagnosis requiring the application of multiple polymorphic markers. We developed PCR design, involving the application of 13 highly polymorphic microsatellite markers, located within the duplicated area and closely linked to PMP22 gene. Seven PGD cycles were performed for CMT type 1A, X1 and 2E. In 2 of 3 couples with paternally derived duplication, single sperms analysis was performed to determine normal and mutant haplotypes. In the remaining 4 PGD cycles performed for 3 CMT1A couples with maternal mutation, both polar bodies (PBs) (24) and blastomeres (22) were analyzed, using markers D17S1357, D17S2229, D17S2226, D17S2225, D17S839, D17S2224, D17S2221, D17S2220, D17S291, D17S2219, D17S2218, D17S2217, D17S2216, which were amplified in multiplex hemi-nested PCR system, followed by fragment analysis. These cycles resulted in transfer of 7 embryos free of PMP 22 duplication, yielding one birth of an unaffected child, and one ongoing singleton pregnancy. Two PGD cycles were performed for the X-linked form of CMT, caused by mutations in connexin-32 gene (Cx32), for which 28 PBs and 2 blastomeres were tested for the presence of the V95M mutation in Cx32 gene, simultaneously with the short tandem repeats (STR) DXS453, DXS8052, DXS8030, DXS559, DXS441, which resulted in transfer of 5 mutation normal embryos failing to yield a clinical pregnancy. The other PGD cycle was performed for maternally derived autosomal-dominant CMT type 2E, caused by mutations in the light polypeptide neurofilament protein gene (NEFL). Testing of 28 PBs and 6 blastomeres for the presence of the mutation P8R, simultaneously with microsatellite marker D8S137 resulted in transfer of 3 unaffected embryos and birth of a child with normal NEFL gene. This is the largest experience of PGD for CMT, demonstrating the reliability and accuracy of the applied PGD designs.

Segregation of mtDNA mutations during embryo-fetal life: implications for genetic counselling. *J. Steffann¹, N. Gigarel¹, C. Bouchet¹, P. Burette¹, N. Frydman², I. Giurgea¹, S. Lebon¹, A. Rotig¹, R. Frydman², G. Tachdjian², S. Masmoudi¹, J.P. Bonnefont¹, A. Munnich¹.* 1) Genetics Dept, Necker Hospital, Paris, France; 2) Gynecology and obstetrics Dept, Beclere hospital, Clamart, France.

Mitochondrial DNA (mtDNA) mutations cause a wide range of serious genetic diseases with maternal inheritance. Due to the high transmission risk and the absence of efficient therapy in these disorders, at risk couples often ask for prenatal and/or preimplantation diagnosis. The few available data point out that predictive value of a prenatal analysis for the severity of the clinical phenotype is questionable. This is likely to be due to tissue and age variation of heteroplasmy. In an attempt to retrace the natural history of mtDNA mutation segregation from conception to adult life, we developed a PCR test enabling quantification for both NARP (Neurogenic weakness, Ataxia, Retinitis Pigmentosa) 8993TG and MELAS (Mitochondrial myopathy, Encephalopathy, Lactic Acidosis, Stroke-like episodes) 3243AG mutations from low amounts of DNA. Using this test, we performed the first preimplantation diagnosis for NARP. Extremely skewed segregation of 8993TG was observed, with a 100% mutant load in one embryo and no detectable mutation in the two others. Comparative analysis of two blastomeres from each embryo did not show any variation of the mutant DNA rate. Both mutation-free embryos were transferred and a singleton pregnancy is ongoing. Result of the test is to be controlled on a next amniotic fluid sample. Additionally, 12 prenatal analysis for MELAS or NARP mutations were carried out from CVS and/or amniocytes. Maternal mutant loads ranged from 0 to 70% in blood. In 14 of 17 fetal DNA samples, the mutant DNA rate was below 10 % or over 90%. When studied, the mutant load was fairly stable at 10, 15, and 30 week- gestation. Maternal mutant load had no predictive value for the fetus status, except for women with no detectable mutant DNA. All children with 20 % of mutant DNA in prenatal period are healthy at 1-6 years of age. Collecting more data on mitochondrial segregation during embryonic and fetal stages should further help interpreting prenatal analysis results.

Improved X-linked Alport syndrome genetic testing to perform preimplantation genetic diagnosis: direct and indirect approaches. *B. Tazon-Vega¹, E. Ars¹, P. Ruiz¹, R. Torra².* 1) Molecular Biology Laboratory, Fundació Puigvert, Barcelona; 2) Nephrology Department, Fundació Puigvert. Barcelona. SPAIN.

Alport syndrome (AS) is characterized by persistent hematuria, progressive renal failure and splitting and thinning of the glomerular basement membrane. Mutations in the *COL4A5* collagen gene are responsible for X-linked AS (XLAS) that accounts for 85% of the total AS familial cases. Preimplantation genetic diagnosis (PGD) applied to patients at risk of transmitting X-linked diseases usually consists of the positive selection of female embryos. However, description of a 10-15% of XLAS female carriers suffering from chronic renal failure before 40-50 years of age has given rise to the necessity of developing a specific PGD for this disease. The purpose of this study is to develop a reliable technique in order to perform an XLAS PGD absolutely independent of the embryo's sex. First, an XLAS family is characterized by linkage analysis on Xq22-q23 followed by a mutation screening of the *COL4A5* gene. To simplify this genetic testing, total RNA is extracted from the propositus' hair-roots and the complete *COL4A5* coding sequence is amplified in 10 cDNA overlapping fragments for direct sequencing. Once a mutation is identified in the propositus and its segregation with the disease is confirmed in the genomic DNA of the family, a direct PGD can be performed. If no mutation is detected, then linkage analysis is the alternative. We have improved the XLAS indirect diagnosis for a PGD application by a co-amplification on a 5pg DNA sample of 4 different microsatellite combinations of 3 markers: 1) *DXS1120*, *DXS6802* and *COL4A5-2B6*; 2) *DXS6797*, *DXS6802* and *COL4A5-2B6*; 3) *DXS6802*, *COL4A5-2B6* and *DXS1210*; and 4) *DXS6802*, *COL4A5-2B6* and *DXS456*. It is possible to choose between these groups of markers depending on their informativity showed in the previous linkage analysis of the family. Currently, we are assessing the efficiency of both, the mutation detection approach and the multiplex-PCR of the 4 microsatellite sets on single buccal cells. Finally, we will test these protocols on blastomeres from non-viable embryos prior to offering the XLAS PGD to interested couples.

Clinical consequences of an increasing trend of preferential use of cultured villi for molecular diagnosis by CVS.

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Chorionic villus sampling (CVS) allows for early prenatal diagnosis and the option of pregnancy termination by D&C at 14 weeks with significantly fewer complications than 2nd trimester termination. A direct diagnosis using intact villi yields quicker results than using villus cells expanded in culture. We examined our molecular CVS cases from 1988-2004: 76 diagnoses utilized DNA from fresh or frozen villi and 37 from cultured villi. Time from CVS to diagnosis, as expected, was longer when cultured cells were used (23.9 days vs 13.2 days for direct extraction) ($p < 0.001$). Average G.A. at diagnosis was 14 5/7 weeks for cases when cultured cells were used vs 13 1/7 weeks with uncultured villi ($p < 0.001$). Excluding 3 cases sampled at 13 4/7 weeks, 17/36 (47%) of results from cultured cells were received at 15 0/7 weeks or later vs only 8/74 (11%) using uncultured villi ($p < 0.0001$). There were no inaccuracies in the molecular diagnoses in our series despite one case of maternal contamination involving cultured cells. We then compared our first 10 years' cases to those from '99-'04. The time from CVS to diagnosis increased from 14.6 days in '88-'93 to 16.6 days in '94-'98, to 17.7 days in '99-'04. During the first decade, reference laboratories required cultured cells for the analysis in 13% of cases vs 55% of cases since 1999 ($p < 0.0001$).

Conclusions: Direct DNA extraction saves ~11 days from time of CVS to results. More women are afforded the option of an earlier and safer pregnancy termination if uncultured villi are used for molecular diagnosis. This advantage and a reduction in the anxious waiting time were forfeited in 47% of our CVS cases in which cultured cells were used. Implementation of standardized DNA extraction protocols and sample size requirements can optimize the use of uncultured villi for molecular prenatal diagnosis. Increased awareness of the importance of a rapid result and the advantages of direct DNA extraction from uncultured villi can lead to improvements that are of clinical significance for patients undergoing early prenatal diagnosis.

Second Trimester Maternal Serum Screening as a Predictor of Adverse Pregnancy Outcomes in a Venezuelan Population. *F. Álvarez Nava, M. Soto, T. Padrón, A. Morales-Machín, M.C. Martínez, A. Rojas-Atencio, K. Urdaneta.* Unidad de Genética Médica, Universidad del Zulia, Maracaibo, Venezuela.

Objective: Abnormal levels of maternal serum alphafetoprotein (AFP), human chorionic gonadotropin (hCG) and unconjugated estriol (uE₃) have been associated to fetal Down syndrome. These abnormal levels have also been implicated in spontaneous miscarriage. The purpose of this study was to examine the relationship between the abnormal maternal serum concentrations of AFP, -hCG and uE₃ in the second trimester and the presence of various adverse pregnancy outcomes in a Venezuelan population. **Study Design:** Maternal serum AFP, -hCG and uE₃ concentrations were measured in 2,621 single-fetus pregnancies from 15 to 21 weeks in Maracaibo, Venezuela. Patients with fetal anomalies or chromosome aberrations were excluded from this study. Serum marker levels were expressed as gestational age-specific multiples of the median (MoMs). The incidence of various adverse pregnancy outcomes such as spontaneous preterm labor, fetal growth restriction, pregnancy induced hypertension, intrauterine fetal demise, oligohydramnios, spontaneous miscarriage and placental abruption were evaluated. **Results:** Of the 2,621 patients in this study, pregnancy complications were observed in 296 (11.29%). There was an increase in the relative risk for spontaneous miscarriage (RR = 6.33; RR = 5.53) at -hCG <0.2 MoM and at uE₃ <0.4 MoM, respectively. In the cases of oligohydramnios, AFP concentrations were significantly increased (RR = 4.18). **Conclusion:** Low levels -hCG and uE₃ are associated with a higher incidence of spontaneous miscarriage and high levels of AFP are increased in oligohydramnios, so second trimester maternal serum screening is a good predictor of adverse pregnancy outcomes.

The majority of fetal nucleated red blood cells in maternal blood are not amenable to analysis by FISH. *T. Babochkina, S. Hristoskova, S. Mergenthaler, G. De Napoli, S. Tercanli, W. Holzgreve, S. Hahn.* University Women's Hospital, University of Basel, Basel, Switzerland.

Introduction: The analysis of fetal nucleated red blood cells (NRBCs) enriched from maternal blood is an attractive alternative for the risk-free prenatal diagnosis of aneuploidies. Current results are discouraging due to low levels of sensitivity or inability to detect fetal NRBCs by FISH analysis. We have previously shown, that single cell PCR on micro-manipulated NRBCs, could be used for the reliable determination of multiple fetal loci. Therefore, a disparity appears to exist between the analysis of fetal NRBCs by PCR and FISH.

Methods: NRBCs were enriched from maternal blood samples by anti-CD71 MACS, identified morphologically by May-Greunwald staining and examined by XY-FISH.

Results: Our analysis indicated that in samples obtained from pregnancies with male fetuses, on average approximately 5 % of the erythroblasts were XY-positive, while almost 50% were clearly of maternal origin as they contained XX-signals. The remaining erythroblasts, however, had aberrant or no FISH signals. In pregnancies with female fetuses on average only 45% of the erythroblasts were XX-positive, while the remaining erythroblasts had aberrant or no FISH signals. These results were not improved using a variety of decondensation strategies.

Conclusions: Our results indicate that only half of the NRBCs could be efficiently analysed by FISH, and that these were most likely of maternal origin, as they had a XX genotype. In this regard, our data parallel our previous PCR analysis which indicated that approximately 50% of the circulatory NRBCs were of maternal origin.

Improved Enrichment of Fetal DNA from Maternal Plasma through targeting of Apoptotic Bodies. *F. Bischoff*¹, *C. Horne*², *D. Dang*¹, *J. Scott*², *J.L. Simpson*^{1,3}, *D.E. Lewis*². 1) Depts OB/GYN; 2) Immunology; 3) and Mol/Hum Genet, Baylor Col Medicine, Houston, TX.

Fetal DNA is present in the maternal circulation, correlating positively with gestational age and showing near 100% sensitivity in detecting fetal sequences. While available techniques suffice for diagnostic studies (e.g. RhD), quantitative estimates of fetal DNA are less reliable. Yet, quantitative analysis of fetal DNA is an attractive modality for identifying pregnancy-related complications (i.e. pre-eclampsia) or for use as a novel analyte in non-invasive prenatal aneuploid screening. Better methods to improve isolation and enrich fetal DNA should logically be based on knowledge of the structural forms in which circulating plasma DNA exists. **HYPOTHESIS:** Fetal DNA circulates pre-dominantly in the form of membrane-bound apoptotic bodies which are amenable to focused enrichment strategies. **METHODS:** Maternal plasma (500 ul) specimens (n=28; mean 15.9 wks gestational) were analyzed prospectively using Acridine Orange (AO) dye to stain DNA followed by flow sorting for positive selection of labeled plasma DNA. DNA was extracted using the Qiagen DNA purification kit. Unaware of fetal status, real-time PCR quantified fetal Y-specific (DYS1) and total (GAPDH) genome equivalents (GEq/mL plasma). **RESULTS:** Testing only 150ul of the plasma processed for flow sorting, fetal DNA was still confirmed in 9 of 15 (60%) samples derived from a pregnancy carrying a male fetus. The mean GEq/ml plasma of fetal Y- and GAPDH sequences were 27.8 and 149, respectively. The ratio of fetal to total DNA was 1 to 6, significantly greater than the 1 to 1000 expected using no enrichment. In the six samples in which Y-sequence was not detected, quantity of GAPDH sequence was less than half the mean (71.3 GEq/ml plasma), indicating that with greater volume sensitivity could near 100%. Of 13 samples in which the fetus was female, one showed Y-sequence but at low quantification of 2.8 GEq/ml. **CONCLUSIONS:** Our results confirm that fetal DNA circulates pre-dominantly in apoptotic bodies and that enrichment based on this knowledge can improve quantitative approaches for clinical application.

MR imaging of the fetal cerebellar vermis *in utero*: description of some useful anatomical criteria for normal development. *S.I. Blaser*¹, *A.J. Robinson*¹, *A. Toi*², *D. Chitayat*², *M. Gundogan*¹, *S. Pantazi*², *S. Laughlin*¹, *G. Ryan*².
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Purpose To produce an atlas of easily identifiable and reproducible measurements and markers of normal anatomic development of the fetal cerebellar vermis *in vivo*.

Â Â Â Materials and Methods Retrospective analysis of the midline sagittal views of the cerebellar vermis was performed in 189 fetal MR studies. Analysis included identification of the fastigial point and vermian fissures, degree of coverage of the fourth ventricle, cerebellar growth and proportions, tegmento-vermian angle, and associated abnormalities of the posterior fossa, brainstem and CNS.

Â Â Â Results Gestational age ranged from 14.0 to 38.6 weeks with a mean of 26.7 weeks. Useful midline sagittal views were obtained in 152 studies, with a total of 230 measurements. Average craniocaudal diameter of the cerebellar vermis follows growth approximately predicted by the linear equation: diameter(mm)=0.74 x gestational age(weeks)-6.11, with an R² value of 0.89. Average height above and below the fastigial point also followed a linear progression, with percentages above and below of 47.9% and 52.1% respectively, and no significant change of this ratio with gestational age. The tegmento-vermian angle was always small in normal fetuses, but was often markedly increased in abnormal fetuses. Coverage of the 4th ventricle should usually have occurred by 17-18 weeks. The declive and primary fissure were always visible in normal fetuses from 17.5 weeks. The other cerebellar vermian fissures were seen at approximately the following gestational ages: secondary at 20 weeks, prepyramidal at 21 weeks and preculmenate at 22 weeks. The other lobules became visible from 24 weeks and most were visible by 27 weeks. Correlation with *in vitro* studies shows a delay of 3-5 weeks in the gestational age at which these features are seen.

MR imaging of the fetus *in utero* I: A practical guide to systematic analysis - Central Nervous System. H.

*Branson*¹, *A. Robinson*¹, *S. Blaser*¹, *A. Toi*², *D. Chitayat*², *M. Gundogan*¹, *S. Pantazi*², *G. Ryan*². 1) Hospital for Sick Children, TORONTO, ON, Canada; 2) Mount Sinai Hospital, TORONTO, ON, Canada.

Purpose Well-established protocols exist for the sonographic examination of the fetus, encompassing differing levels of detail depending on the overall risk of the individual pregnancy (i.e., from a routine screening examination up to a tertiary-level detailed assessment of a high-risk pregnancy). No such protocols are established yet for fetal MR imaging. In our institution we have attempted to establish a protocol for reporting fetal MR examinations, in order that a systematic and methodical approach is adopted by our radiologists, including those who are not used to reading these examinations, and to avoid missing important anatomical abnormalities.

Materials & Methods Retrospective analysis was performed in over 190 consecutive fetal MR examinations performed for CNS and non-CNS indications.

Results Analysis included, but was not limited to, biometry, including biparietal diameter, transcerebellar diameter, ventricular atrial width, craniocaudal diameter of the cerebellar vermis, the presence of the cavum septum pellucidum, corpus callosum and other midline and lateral structures, cortical development and appearance of sulci and major fissures, and assessment of the spine.

Conclusion We would like to demonstrate a practical guide to analysis of the central nervous system of the fetus in utero, including how to perform the biometric measurements, and examples of normal and abnormal biometry and development. (Part 1 of 2.).

MR imaging of the fetal eyes: Normal and abnormal development. *D. Chitayat¹, A. Robinson², S. Blaser², A. Toi¹, M. Gundogan², S. Pantazi¹, G. Ryan¹.* 1) Mount Sinai Hospital, TORONTO, ON, Canada; 2) Hospital for Sick Children, TORONTO, ON, Canada.

Purpose To define easily identifiable and reproducible measurements of normal anatomic development of the fetal eyes *in vivo*. Current measurements of binocular and intraocular distances are defined by ultrasound and are measured according to the bony landmarks of the medial and lateral orbital walls. These bony landmarks cannot be seen on fetal MR, and therefore these ultrasound measurements cannot be directly applied to MR imaging, however the vitreous is well-defined.

Materials and Methods Retrospective analysis of the coronal and axial views of the orbits was performed in 197 consecutive fetal MR examinations performed for CNS and non-CNS indications. Analysis included measurement of the vitreous diameters, intraocular distance and binocular distance. These were plotted against gestational age. Fetuses with abnormalities affecting the eyes were evaluated separately.

Results Normal growth of ocular diameter, intraocular distance and binocular distance is demonstrated, with direct correlation to measurements made by ultrasound at the same gestational age. Additionally, examples of abnormal growth and development are shown including hypertelorism, hypotelorism, microphthalmia, anophthalmia, persistent hyperplastic primary vitreous, and cyclopia, with underlying diagnoses such as frontonasal dysplasia, septo-optic dysplasia holoprosencephaly spectrum, Matthew-Wood, Walker-Warburg, Cat-Eye and Pfeiffer syndromes, and trisomy 22.

The need for fetal karyotyping in anencephaly: recurrent anencephaly due to a familial unbalanced (3;5) translocation. *K.L. Ciprero¹, J.D. Hoffman¹, D.A. Driscoll¹, M. Sagi², N.B. Spinner¹, E.H. Zackai¹.* 1) Div Human Gen & Molec Biol, Children's Hospital of Philadelphia, Philadelphia, PA; 2) Hadassah Medical Org., Jerusalem, Israel.

Prenatal diagnosis of neural tube defects (NTDs) has been greatly facilitated by maternal serum screening programs and improved sonographic techniques. Although most NTDs are shown to be multifactorial, the etiology remains heterogeneous. Recurrence risks as well as risk for other disorders and subsequent pregnancy monitoring depend on the underlying cause. Support for fetal karyotyping has come from surveys of aneuploidy among prenatally detected NTDs (7%-Hume, 1996). There are reports of recurrent anencephaly with trisomy 2p23.2-2pter with additional evidence from single cases that 2p24 is involved. Here we report another possible locus in a three-generation family with five cases of anencephaly. One of these has proven to have an unbalanced translocation with monosomy 3q26.2-3qter and trisomy 5p15.1-5pter, secondary to a familial balanced (3;5) translocation. In generation I, there was an anencephalic male whose healthy male sibling has a (3;5) balanced translocation. The latter is the father of two male fetuses with anencephaly and five healthy children. Three of these children are balanced carriers, one of whom had a child with multiple congenital anomalies with a 46,XX,der(5),t(3;5)(q26.2;p15.1) karyotype, consistent with monosomy 5p15.1-5pter and trisomy 3q26.2-3qter. The other two children each had a fetus with anencephaly, one of which was proven to have a 46,XX,der(3),t(3;5)(q26.2;p15.1) karyotype. We wish to emphasize the importance of fetal karyotyping in cases with NTDs. In addition, the anencephalic cases in our report suggest that a gene/genes in one of these chromosomal regions are associated with NTDs, especially since parietal meningocele has been previously reported in an infant with a 3q27-3qter deletion.

Increased nuchal translucency: A marker of fetal parvovirus? *J. Furnival*¹, *G. Ryan*², *J. Kingdom*², *A. Toi*³, *S. Keating*⁴, *R. Tellier*⁵, *W. Meschino*¹. 1) Genetics, North York General Hospital; 2) Fetal Medicine Unit; 3) Medical Imaging; 4) Pathology, Mount Sinai Hospital; 5) Microbiology, Hospital for Sick Children; Toronto, ON, Canada.

A 36 year-old G3P2 woman was referred to the Prenatal Genetics Clinic at 13 weeks gestation with an increased nuchal translucency (NT) measuring 3.4 cm. Following genetic counselling, an amniocentesis was done revealing normal chromosomes. Fetal ultrasound at 19⁺⁶ weeks showed cardiomegaly, a pericardial effusion and ascites. One week later a tachyarrhythmia was noted. In retrospect, the patient recalled an indirect exposure to Fifth disease midway through her first trimester. There was an outbreak in her son's daycare, but he remained asymptomatic. She also recalled having a period of extreme fatigue between 8 to 10 weeks gestation with no other symptoms. Human parvovirus B19 was detected by PCR from the reserved amniotic fluid. Cardiac function and hydrops worsened over the next 48 hours and the fetus became moribund. The pregnancy was terminated because of the grave prognosis. Fetal pathology confirmed hydrops fetalis. Hematopoietic cells and endothelial cells in the fetal heart, liver and brain showed characteristic inclusions and stained positively for parvovirus on immunohistochemistry. The finding of increased NT with a normal karyotype raises the risk for a variety of structural abnormalities. Fetal parvovirus has been associated with increased NT in 2 previous cases as well as 3 cases of first-trimester hydrops fetalis. Each resulted in the birth of a healthy infant. Several other first trimester cases had early hydrops and fetal demise. First trimester parvovirus infections have also been associated with spontaneous abortion and intrauterine growth restriction. The finding of an increased NT should alert the practitioner to the possibility of later development of hydrops, including that secondary to fetal parvovirus. Appropriate investigations with serology, serial ultrasounds, and possibly PCR testing should be undertaken, particularly if a positive history is revealed.

Prenatal diagnosis of chromosomal defects in Tunisian population. *D. H'MIDA, H. ELGHEZAL, S. MOUGOU, H. SANNANA, A. SAAD.* Cytogenetics, Farhat Hached Hospital, Sousse, Tunisia.

Objective:Increasing annual number of prenatal karyotypes carried out in our laboratory indicates that prenatal diagnosis of chromosomal abnormalities has become a large activity in handicap prevention policy in our country. In our study we report indications and results of foetal karyotypes carried out in Tunisian population during the period of 1993-2003.**Material and Methods:**During the study period, 4765 pregnant women were referred to our center for prenatal diagnostic of chromosomal abnormalities. Foetal karyotypes were performed after amniotic liquid culture according to conventional techniques.Five classical contexts indicate prenatal cytogenetic analysis: advanced maternal age, sonographic detection of foetal malformation, nuchal translucency, maternal serum markers and parental chromosomal abnormalities.**Results:**Two hundred and four chromosomal abnormalities were detected (4.2%). Autosomal abnormalities represent 81.86% of the total chromosome anomalies. Among them, trisomy 21, followed by trisomy 18 and 13 are the most frequents. Sex chromosome abnormalities constitute 18.13% of the total chromosome anomalies. Advanced maternal age (>36 years) was the most common indication (50%). However, nuchal translucency; representing the indication of chromosomal prenatal diagnosis only in 2.4% of cases; appears to have a high predictive value. Maternal serum screening represents a rare indication in our series.**Discussion:**Prenatal diagnosis of chromosome anomalies is accepted by patients and became available in routine in Tunisia as in occidental countries. However, indications and results are not similar. Advanced maternal age persist to be the most common indication. Nuchal translucency screening test and maternal serum screening represents rare indications in spite of their higher predictive value.

Prenatal Cytogenetic Diagnosis on 47,730 Amniotic Fluid Specimens. *R. Habibian¹, S. Nawaz², A. Hajianpour¹.* 1) Genzyme Genetics, Pasadena, CA; 2) USC School of Pharmacy, LA, CA.

Habibian Rezvan, Nawaz Shahbanoo, and Hajianpour Atieh Genzyme Genetics, 11 W. Del Mar Blvd, Pasadena Ca 91007A total of 47,730 amniotic fluid specimens were received at Alfigen/The Genetics Institute between the years of 1996 and 1999. The leading indications for referral were advanced maternal age (68.6%), serum marker Down Syndrome risk (10.6%), abnormal ultrasound (9.64%), and high MSAFP (3.23%). Of these 47,730 specimens, 10 specimens were cancelled, 8 specimens were for confirmation study and 92 specimens failed to grow (0.19%). The adjusted growth failure rate was 0.05% (excluding fetal demised and contaminated specimens). The average turnaround time was 7.90 days. Overall, 92.2% had a turnaround time of ten days or less. Approximately 75.29% of our cases were received by couriers on the same day of specimen collection, while the remaining 24.71% were received by overnight express mail. A total of 47,620 cases, representing 99.77% of the total amniotic fluid specimens received were used for the following data analysis. The abnormality rate, excluding inv(9), was 1582/47,620 (3.32%). Of the 1582 abnormal cases, 1230 cases (77.75%) were autosomal chromosome abnormalities; 278 cases (17.57%) were sex chromosome abnormalities; 42 cases (2.66%) had marker chromosomes; 22 cases (1.39%) were polyploidy, with 21 triploidies and one tetraploidy; and 10 cases (0.63%) had reportable variant chromosomes. The total number of mosaic abnormalities in all five major groups were 221/47,620 cases (0.46%). Of the autosomal chromosome abnormalities, 79.06% consisted of numerical abnormalities, and 20.95% consisted of structural abnormalities. Of the sex chromosome abnormalities, 88.13% were numerical abnormalities and 11.87% were structural abnormalities. Multiple cell pseudomosaicism was observed in 0.15% of the amniotic fluid results. Detailed data analysis will be presented.

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Folic Acid Peri-Conceptional Supplementation: Positive Associations with Miscarriage. *E.B. Hook.* School of Public Health, University of California, Berkeley, CA 94720-7360.

In data on the association of folic acid peri-conceptional supplementation with loss of conceptions before 20 weeks Czeizel et al (*Arch Gynecol Obstet* 255:131,1994) observed a 16% increase, ($P < .05$, with 95% confidence limits of 1% to 30% increase). (See also Hook & Czeizel *Lancet* 350: 515, 1997; Hook *Am J Med Genet* 92: 301, 2000; and 104: 181, 2001). This analysis considers other reports pertinent to this association. Wald (*Ann NY Acad Sci* 678:112, 1993) interpreted MRC data as in essence indicating no association with miscarriage, but in data presented on this point (p. 126) the increase was 15%. Reanalysis of MRC data co-authored by Hackshaw indicated an increase of 6% not 15%, (not significant, but with 95 % upper confidence limit of 43% increase). In data of Windham et al. (*Am J Med Gen* 90: 261, 2000) the increase is 14%, $P = < .10$, with 95% confidence limits of 4% decrease to 34% increase. In all three there is an increase and none exclude an increase as high as 30%. Authors of the recent and largest study from Jiaying City, China, Gindler et al 2001 (*Lancet* 358: 796) interpret their data as indicating no association of miscarriage with any folic acid supplementation, indeed a 3% decrease and reject a 12% or greater possible increase. But from data they present one may calculate that those with *complete* peri-conceptional supplementation have about a 15% *increase* in miscarriage ($P < .001$, confidence interval of 7% to 25% increase), in comparison with those with *no or incomplete* supplementation (the latter including those with just trivial usage). However analyzed, *all* studies of supplementation are consistent with an increase as high as 12% and suggest strongly that folic acid *peri-conceptional* supplementation is associated with about a 15% increase in detected pregnancy loss (with likely limits of 7% to 25% increase). Such association may be due to folic acid increasing the *recognition* of such loss not in its *induction*.

Molecular Nature of Nucleic Acid Containing Bodies in Maternal Plasma. *C. Horne¹, A. Orozco¹, J. Scott¹, D. Dang², J.L. Simpson^{2,3}, D.E. Lewis¹, F.Z. Bischoff².* 1) Immunology; 2) OB/GYN; 3) Hum/Mol Genet, Baylor Col Med, Houston, TX.

Cell-free fetal DNA is present in the maternal circulation, however; the biological form in which this DNA exists and the mechanisms underlying its quantitative variation in plasma are unclear. **HYPOTHESIS:** We hypothesize that the majority of fetal DNA circulates in apoptotic bodies. Further elucidating the fundamental properties of these apoptotic bodies will enable better recovery and enrichment of DNA from these bodies. **METHODS AND RESULTS:** Maternal plasma (500ul) from 10-16 wks gestation was stained with acridine orange (AO), a nucleic acid dye. Using a Coulter XL flow cytometer, the resulting light scatter (LS) pattern suggested numerous sub-cellular particles bound to nucleic acids and scattered more light than control intact cells. To verify that the particles were not whole cells, maternal plasma was filtered through a 0.2 micron filter before staining, resulting in no change in LS or in DNA fluorescence. Additionally plasma was subjected to high-speed centrifugation (14,000xg) prior to AO staining. This treatment resulted in physical separation of the particles. That DNA fluorescence also decreased after centrifugation, indicated that the nucleic acids can be physically separated into smaller fragments. To further investigate the nature of these bodies, maternal plasma was treated with nucleases, proteinases, and detergents before staining with AO. After proteinase K treatment, the LS pattern was nearly destroyed, indicating membrane disruption. As expected, the bulk of the DNA precipitated out of solution. However, after treatment with 1% SDS, the number of events within LS gates significantly increased, as did the DNA fluorescence. In contrast, DNase and RNase treatments affected neither the LS pattern nor the fluorescent intensity, demonstrating membrane protection. **CONCLUSIONS:** Circulating cell-free DNA in maternal plasma is stable within apoptotic bodies, which serve to protect DNA from enzymatic treatment. Fetal DNA enrichment strategies can isolate fetal DNA as membrane bound bodies.

Prenatal diagnosis of Marshall syndrome by targeted sonography. *D. Iacoboni¹, BM. Karpel¹, A. Shanske², RW. Marion², SJ. Gross³.* 1) The Center for Fetal Diagnosis and Reproductive Genetics, Department of OB/GYN, North Central Bronx Hospital/North Bronx Health Network, Bronx, New York; 2) The Center for Congenital Disorders, Department of Pediatrics, Montefiore Medical Center/Albert Einstein College of Medicine, Bronx, New York; 3) Division of Reproductive Genetics, Department of OB/GYN, Montefiore Medical Center/Albert Einstein College of Medicine, Bronx, New York.

Background: Marshall syndrome (MS) is a rare, autosomal dominant syndrome characterized by ocular hypertelorism with shallow orbits, craniofacial anomalies, sensorineural hearing loss, and ectodermal dysplasia. Splicing mutations of the 54-bp exons in the C-terminal region of the COL11A1 gene have been identified in some individuals with MS (Annunen et al., 1999). Below, we present the anatomic features obtained from mid-trimester detailed fetal sonogram that was subsequently utilized for prenatal diagnosis of this syndrome.

Case Report: A 24-year-old, G5P5005, African American woman affected with Marshall syndrome presented to the reproductive genetics unit at 25 weeks of gestation. She is a member of a seven-generation family with six known affected individuals (Shanske et al., 1997); molecular analysis failed to identify a mutation in this family. Targeted anatomy was performed and revealed borderline increased intra-ocular distance and a flat profile with a wide and depressed nasal bridge. Remaining fetal anatomy was unremarkable and amniotic fluid volume was within normal limits. The patient received genetic counseling and informed that the findings were consistent with MS. She delivered a fullterm male infant and the diagnosis of MS was confirmed in the newborn period.

Conclusion: Targeted sonography with particular attention to subtle facial features is an important modality in the prenatal diagnosis of MS, particularly in families where the causative mutation remains unknown.

Time for reevaluation of intrauterine survival after prenatal diagnosis of Turner syndrome and non-immune hydrops. *K. Martin¹, J. Garcia-Heras²*. 1) Dept Ob/Gyn, Washington Univ in St. Louis, St Louis, MO; 2) Dept Ped, Washington Univ in St. Louis, St. Louis, MO.

We report 4 cases of non-mosaic Turner syndrome presenting prior to 24 weeks gestation with large, septated cystic hygromas (CH) and subcutaneous edema, with/out pericardial/pleural effusion/ascites. All cases were seen within the past 3 years. The families were counseled regarding the poor prognosis associated with this prenatal diagnosis and elected to continue the pregnancies. One fetus suffered intrauterine demise prior to 24.0 weeks. The other three fetuses demonstrated progressive resolution of the ultrasound findings and were live born at term. All livebirths had features consistent with the diagnosis of Turner syndrome and two of the three livebirths were confirmed to have congenital heart defects. Surerus et al (Ultrasound Obstet Gynecol,2003) conclude that "Turner's syndrome . . with hydrops has an extremely poor prognosis". A case report of survival of a fetus with Turner syndrome, CH and hydrops was published by Mostello et al (Obstet Gynecol,1989). Suchet et al (Can Assoc Radiol J,1992) describe survival in 1 of 4 hydropic fetuses with Turner syndrome diagnosed prenatally. A literature review demonstrates that the majority of pregnancies in which these finding were made underwent termination of pregnancy. Including our series, a total of 14 fetuses with Turner syndrome, CH and hydrops were identified. Eight were liveborn in the third trimester and six (43%) survived. Families faced with the prenatal diagnosis of Turner syndrome, CH and hydrops must be made aware of the possibility of spontaneous resolution and survival. The likelihood of livebirth associated with this finding cannot be absolutely determined, but may be as high as 43%.

Prenatal diagnosis of Budd-Chiari syndrome. *W. Meschino¹, J. Furnival¹, W. Sirkin², J. Wortsman³.* 1) Dept Genetics; 2) Dept Laboratory Medicine; 3) Dept Diagnostic Imaging, North York General Hosp, Toronto, ON, Canada.

Budd-Chiari syndrome (BCS) is characterized by functional and structural abnormalities in the liver resulting from obstruction of hepatic venous outflow. The acute syndrome is usually associated with an underlying thrombophilic process, whereas chronic BCS may be due to vascular pathology. It occurs very rarely in children. We report the first case of fetal BCS to be detected prenatally. The patient is a 32 year old, G2P1 woman of East Indian ancestry who was referred with a positive maternal serum screen for Down syndrome (1 in 8 risk) and progressive fetal ascites at 18 weeks gestation. Other ultrasound findings included polyhydramnios and an unusual pattern of fetal blood flow into the liver. Investigations revealed normal chromosomes on amniocentesis and negative fetal infection studies. The pregnancy was terminated at the parents' request. External examination of the fetus revealed mild facial dysmorphism, a narrow thorax, marked ascites, brachydactyly and a hypospadias. Internal examination revealed dilated hepatic sinusoids and a membranous obstruction of the inferior vena cava (MOVC) occluding greater than 90% of the lumen. MOVC is the single largest cause of BCS (40%) in Asian series, but is much less common in Western cases. Controversy exists as to whether the membrane is a primary malformation or whether it arises from an organizing thrombus. Maternal and paternal thrombophilia studies including Factor V Leiden and antithrombin 3 were normal in our case. The presence of dysmorphic features and congenital anomalies in our case suggest that the MOVC arose as a developmental defect rather than an organizing thrombus. Together these features may represent a new genetic syndrome.

Prenatal Diagnosis in Severe Methylenetetrahydrofolate Reductase Deficiency: Combined Biochemical and Linkage Approach. *C. Morel¹, P. Scott¹, E. Christensen², D.S. Rosenblatt¹, R. Rozen¹*. 1) Depts of Human Genetics and Pediatrics, McGill University, Montreal, Canada; 2) Dept of Clinical Genetics, Rigshospitalet, Copenhagen, Denmark.

Severe methylenetetrahydrofolate reductase (MTHFR) deficiency (MIM 236250) is characterized by varying degrees of developmental delay, motor/gait abnormalities and seizures. More than 85 patients with this condition have been reported. Biochemical abnormalities include homocystinuria, hyperhomocysteinemia and low/normal plasma methionine levels. The degree of severity correlates with residual enzyme activity. Severely affected patients die in infancy or early childhood. MTHFR activity can be assayed in cultured fibroblasts, amniocytes and chorionic villus cells (CVCs). Although close to 50 mutations have been identified in patients with this condition, they tend to be private, therefore limiting the use of direct mutation detection for prenatal diagnosis. Nine polymorphisms have been identified in MTHFR, thus making prenatal diagnosis by linkage analysis a reasonable option, with or without identification of the deleterious mutations. Two of these polymorphisms, 677CT and 1298AC, are each found in the homozygous state in about 10% of a population of European origin. We report the prenatal diagnosis for severe MTHFR deficiency in six at-risk pregnancies. We studied MTHFR activity in cultured amniocytes or CVCs and performed linkage analysis for the 677CT or 1298AC polymorphisms within the MTHFR gene. Enzyme activity was assessed in cultured amniocytes in four pregnancies and ranged from 1.4-6.5 nmols CHO/mg prot/h (U) (control 3.1-9.6U). In two pregnancies, CVCs demonstrated MTHFR enzyme activity of 3.6U and 7.7U (control 4.5-7.8U). These values were compared to the probands who had a range of 0-0.7U (control 9.6-11.7U) in cultured fibroblasts. Linkage analysis for the 677CT or 1298AC polymorphisms predicted that four of the fetuses were heterozygotes and two, homozygous unaffected. The data from both methods were concordant and predicted unaffected fetuses. We suggest that linkage analysis for severe MTHFR deficiency is a practical approach for prenatal diagnosis.

MR Imaging of the Fetus *in utero* II: A Practical Guide to Systematic Analysis - and the rest (non-CNS). S.

*Pantazi*¹, *A. Robinson*², *S. Blaser*², *A. Toi*¹, *M. Gundogan*¹, *D. Chitayat*¹, *G. Ryan*¹. 1) Mount Sinai Hospital, TORONTO, ON, Canada; 2) Hospital for Sick Children, TORONTO, ON, Canada.

Purpose Well-established protocols exist for the sonographic examination of the fetus, encompassing differing levels of detail depending on the overall risk of the individual pregnancy (i.e., from a routine screening examination up to a tertiary-level detailed assessment of a high-risk pregnancy). No such protocols are yet established for fetal MR imaging. In our institution we have attempted to establish a protocol for reporting fetal MR examinations, in order that a systematic and methodical approach is adopted by our radiologists, including those who are not used to reading these examinations, and to avoid missing important anatomical abnormalities.

Materials & Methods Retrospective analysis of was performed in over 190 consecutive fetal MR examinations performed for CNS and non-CNS indications.

Results Analysis included, but was not limited to, evaluation of thoracic and abdominal situs, lung parenchyma, diaphragms, liver and gallbladder, stomach, kidneys (including biometry), bladder, cord insertion, cord vessels, placental site and morphology, and amniotic fluid volume.

Conclusion We would like to demonstrate a practical guide for the analysis of the rest of the fetus (non-CNS), including how to perform the biometric measurements, and examples of normal and abnormal development. (Part 2 of 2.).

Rapid prenatal diagnosis of Trisomy 21 using quantitative fluorescent PCR in uncultured amniocytes. *S.Y Park¹, M.H. Lee¹, D.J. Kim¹, B.Y. Lee¹, E.H. Cho¹, M.Y. Kim², J.H. Yang², J.S. Choi², H.K. Ahn², H.M. Ryu^{1,2}.* 1) Lab Medical Genetics, Samsung Cheil Hosp, Seoul, Korea; 2) Department of Obstetrics and Gynecology, Sungkyunkwan University School of Medicine, Seoul, Korea.

Rapid prenatal diagnoses of Down syndrome have been studied using quantitative fluorescent PCR assays by small tandem repeat (STR) markers. The purpose of our study was to investigate the accuracy for rapid prenatal detection of Down syndrome using the quantitative fluorescent PCR in uncultured amniocytes. Blind prospective study was performed in 905 amniotic fluid samples. All samples were subsequently analyzed by traditional karyotyping. Trisomy 21 was identified in five samples. The informative rate for at least two STR markers was 98.7%. The amplification failure rate was 0% with our current protocol. The uninformative rate by maternal cell contamination (MCC) and inconclusive results were 0.4%, 2.3%, respectively. There were no false-positive or false-negative results. The sensitivity, specificity, and efficiency of the assay for detecting Down syndrome were 100%. QF-PCR for the rapid identification of fetus with trisomy 21 is a reliable, accurate, and speedy technique. This method is particularly valuable for minimizing parental anxiety in the wait for a diagnostic test result.

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MR imaging of the fetal cerebellar vermis *in utero*: Criteria for abnormal development, with ultrasonographic and clinicopathologic correlation. A.J. Robinson¹, S.I. Blaser¹, D. Chitayat², A. Toi², W. Halliday¹, M. Gundogan¹, S. Pantazi², G. Ryan². 1) Hospital for Sick Children, TORONTO, ON, Canada; 2) Mount Sinai Hospital, TORONTO, ON, Canada.

Purpose We previously produced an atlas of easily identifiable and reproducible measurements and markers of normal anatomical development of the fetal cerebellar vermis *in vivo* from 17.5 weeks gestational age to term. This new study was to demonstrate easily identifiable and reproducible measurements and markers of abnormal development, with ultrasonographic and clinicopathologic correlation. Virtually all previous studies of development of the cerebellum have been performed on fetal specimens, and few *in vivo* studies discuss development of the cerebellar vermis *per se*.

Material & Methods Retrospective analysis of the midline sagittal views of the cerebellar vermis was performed in 189 consecutive fetal MR examinations performed for CNS and non-CNS indications. Analysis included identification of the fastigial point and vermian fissures, degree of coverage of the fourth ventricle, cerebellar growth and proportions, tegmento-vermian angle, and associated abnormalities of the posterior fossa, brainstem and CNS.

Results Gestational age ranged from 14.0 to 38.6 weeks with a mean of 26.7 weeks. Useful midline sagittal views were obtained in 152 studies; approximately one quarter had abnormalities affecting the posterior fossa. These included fetuses within the Dandy-Walker spectrum which we subdivided according to presence of dysplasia or hypoplasia, abnormal tegmento-vermian angle, size of cisterna magna, and associated CNS abnormalities. Correlation with MR and US images is demonstrated in addition to clinicopathologic and genetic diagnoses where available.

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Effects of prenatal diagnosis on the birth prevalence of children with chromosomal abnormalities. *M.P. ROTH, B. DOTT, C. STOLL.* Genetique Medicale, Faculte de Medecine, Strasbourg, France.

The objectives of this study were to describe the impact of prenatal diagnosis on the birth prevalence of chromosomal abnormalities during 23 years in a well defined population. The material for this study came from the analysis of data from multiple sources on births and terminations of pregnancy (TOP) after prenatal diagnosis of chromosomal abnormalities in 321,204 consecutive pregnancies of known outcome. The study period was divided into 3 subgroups 1979-1988, 1989-1996 and 1997-2002. In the area under study prenatal diagnosis of chromosomal abnormalities is offered free of charges to all women >38 years. Maternal serum screening (triple test) is offered, free of charges, to all pregnant women since 1997 and fetal ultrasonographic scanning is routine practice. Between 1979-1988, 1989-1996 and 1997-2002 prenatal detection of chromosomal abnormalities increased from 10.5%, 59.2% and 75.0%, respectively. TOP increased in the same proportions during the 3 time periods. The birth prevalence per 10,000 of Down syndrome was 8.89, 9.50, and 4.98, respectively. However the total prevalence of Down syndrome was 11.51, 19.68 and 19.93, respectively. Therefore no significant decline in the total prevalence of Down syndrome was observed. The main reason for these observations is the increase in maternal age, from 24.8 to 30.1. In conclusion the introduction of routine prenatal diagnosis has resulted in a significant fall in the birth prevalence of children with chromosomal abnormalities, but not in the total prevalence of the fetal chromosomal aberrations. More efforts on the primary prevention of chromosomal abnormalities are needed.

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No evidence of fetal DNA in maternal serum after twin pregnancy. *O. Samura, N. Fujito, N. Miharuru, M. Hyodo, M. Tanigawa, K. Ohama.* Dept OB/GYN, Hiroshima Univ Sch Medicine, Hiroshima, Hiroshima, Japan.

Fetal DNA in maternal blood has been used for non-invasive prenatal diagnosis. However, in a recent report fetal male DNA was detected in the plasma of 22% healthy women with previous sons for up to several decades after delivery. We investigated short term persistence of fetal DNA in maternal serum of twin pregnancy after delivery. Twenty seven twin pregnant women who had at least one male fetus were recruited. All of the women gave their written consent after being informed about this study. Maternal venous blood samples were taken before delivery. After delivery of the baby, 7ml maternal peripheral blood was collected at 30 minutes and 1 day postpartum. The amount of cell-free fetal DNA in maternal serum was quantified by calculating the concentration of the SRY gene sequence from the Y chromosome using taqman PCR. Median concentration of fetal DNA in serum was 153.21 copies/ml (Range 16.93-485.39 copies/ml) before delivery and 102.6 copies /ml (Range 0-391.62 copies/ml) 30 minutes after delivery. Fetal DNA was detected in only one case 24hrs after delivery. Molecular microchimerism due to circulating fetal DNA persisting from previous pregnancies should not hamper non-invasive serum-based prenatal testing.

Trisomy 17 mosaicism cases diagnosed prenatally. *G. Sekhon, B. Haas, M. Fernandez, K. Thornell, K. Collen.*
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Mosaicism for trisomy 17 in prenatal diagnosis is an uncommon finding, while postnatal cases are exceptional. Trisomy 17 mosaicism remains an uneasy situation for genetic counseling and for a decision during pregnancy. Limited medical literature has addressed the clinical significance of true CVS or amniotic fluid trisomy 17 mosaicism. Such mosaicism is more commonly of extra-embryonic origin and often associated with normal fetal development. Prior reviews have reported at least two unfavorable outcomes out of twenty prenatally diagnosed reported cases with trisomy 17 mosaicism. Although post-natal lymphocyte studies were normal in all such cases, investigators observed trisomy 17 mosaicism on cultured skin fibroblasts only in the two adverse cases. Between September 1994 and June 2004, Genzyme Genetics has diagnosed twenty (20) prenatal cases with trisomy 17 mosaicism and no additional cytogenetic anomalies. We have contacted the referring obstetricians of each case inquiring about the decision reached by the patient about continuation of the pregnancy and any additional factors besides the mosaicism that might have led to a decision of termination. The obstetricians of those cases continuing the pregnancy were asked to provide obstetrical complications, delivery details including gestational age, as well as standard newborn evaluation measurements including length of stay prior to discharge from the nursery or NICU. In addition, the referring obstetrician is asked to provide the mother of the child an invitation to participate in the survey by giving her and the child's pediatrician an evaluation form regarding the child's health. [The age range for this group of children is neonate through age 10]. To date we have gotten confirmation of a child's health from five of the twenty cases. All five are healthy and phenotypically normal infants. Additional responses are anticipated.

Prenatal detection of cystic hygroma in a pregnancy with an unbalanced translocation involving 6q monosomy and 4q trisomy. *E. Story, S. Petersen, K. Murphy, M. Spencer, K. Blakemore, G. Stetton.* Department of Gynecology & Obstetrics, Johns Hopkins Hospital, Baltimore, MD.

An unbalanced chromosome translocation is an uncommon finding with fetal cystic hygroma. We report a case of an unbalanced translocation ascertained by chorionic villus sampling at 12 6/7 weeks following a first trimester nuchal translucency ultrasound that revealed a fetal septated cystic hygroma. G-band analysis and subtelomere probe studies revealed a karyotype of 46,XX,der(6)t(4;6)(q27;q25). Parental karyotypes indicated that this chromosome rearrangement was de novo. Fetal demise in utero occurred at 14 3/7 weeks gestation.

Lymphatic obstructions resulting in an enlarged nuchal translucency of greater than 3.0 mm or septated cystic hygroma are present in approximately 0.8% of fetuses evaluated between 10 and 16 weeks gestation. The vast majority of those with a septated cystic hygroma will have an abnormal karyotype, most often monosomy X, or trisomy 21, 18, or 13. Both trisomy 4q and monosomy 6q have been described in viable offspring, although not concomitantly. Viable offspring with partial trisomy 4q have been reported to have psychomotor and mental retardation, seizures, growth restriction, short neck, dysmorphic facies, and major anomalies. Viable offspring with partial monosomy 6q have been reported to have mental retardation, retinal abnormalities, postnatal growth retardation, dysmorphic facies, and webbed neck. In addition to this case, cystic hygroma has been reported in one other case of 6q25-6qter monosomy. Postnatal webbed neck in individuals with partial monosomy 6q may represent a resolved cystic hygroma.

Given the unbalanced karyotype in this fetus with a cystic hygroma, it is possible that there may be several candidate genes involved in lymphatic development in the 6q25-6qter and/or 4q27-4qter regions. Two genes of interest in 6q25-6qter that have been identified are: Vasoactive Intestinal Peptide, which has been implicated in the regulation of lymphatic pumping activity; and the T-box gene *Brachyury*, which is involved in posterior mesodermal development.

Mosaicism in CVS revisited. *M. Thangavelu, B. Huang.* Genzyme Genetics, Orange, CA.

Most chromosome mosaicisms observed in CVS is reported to be confined to the placenta, with the incidence of confined placental mosaicism being 1%. Follow up amniocentesis or cordocentesis may be performed to confirm fetal origin of the mosaicism. We analyzed cytogenetic data on CVS analyzed at Genzyme Genetics to determine (1) the frequency of mosaicism for various chromosomal abnormalities and (2) the possibility of confirming the abnormalities in follow up investigations. Mosaic abnormalities were observed in 315 of 26,306 (1.2%) specimens investigated. Mosaicism for trisomy 2 was the most common mosaic abnormality (47 cases; 14.9% of mosaic cases). Mosaics for 45,X was the next most frequent abnormality (45,X/46,XX in 9.2% and 45,X/46,XY in 7.6%). This was followed by mosaicism for trisomy 7 (7.3%), trisomy 18 (6.7%), trisomy 8 (5.1%), trisomy 15 (4.1%), trisomy 9 (3.8%) and trisomy 12 (2.9%). Mosaicism for a marker chromosome and mosaicism for structural abnormalities were observed in 5.1% and 7.6% of cases with mosaicism, respectively. Mosaicism for complex and multiple aneuploidies was observed in 25 (7.9%) cases. Data on follow up analysis was available for 166 cases. The following mosaic abnormalities were confirmed: 45,X/46,XY (6/13), 45,X/46,XX (3/8), +21 (3/4), +18 (2/19), +7 (1/11), +8 (1/8), +9 (1/6), +12 (1/8), +13 (1/6), +14 (1/3), and tetraploidy (1/1). The marker was confirmed in 2/7 cases. Mosaicism for +2 was not confirmed in any of the 24 cases that had follow up studies. Of the 166 cases that had follow up investigations, the abnormality was confirmed in 27 (16.3%). Consistent with previous studies, our data suggests that mosaicism for certain abnormalities observed in CVS may indeed be true fetal mosaicism. It is therefore important to recommend follow up studies, particularly for those abnormalities with a higher probability of being present in the fetus. Our data also suggests that the probability of fetal mosaicism varies depending on the abnormality. Knowledge of this probability is important in determining (1) how exhaustive the follow up analysis ought to be and (2) how to interpret the results of the follow up studies, particularly when mosaicism is not confirmed.

Prenatal Finding of Trisomy 22. *M. Thomas¹, A. Toi¹, S. Keating², D. Chitayat¹*. 1) Dept Medical Genetics, Mount Sinai Hosp, Toronto, ON, Canada; 2) Dept Pathology, Mount Sinai Hosp, Toronto, ON, Canada.

We report a case of a fetus with a karyotype of trisomy 22 detected prenatally. This was a second pregnancy to a healthy couple who are non-consanguineous and of Caucasian origin. First trimester dating ultrasound was reported as normal. No first or second trimester screening was performed. Second trimester ultrasound was not done. Ultrasound at 35.7 week gestation reported a fetus of 30.9 weeks size with large 4th ventricle and cleft of lower vermis, cleft lip and primary palate, hypertelorism, flat profile, small stomach, increased amniotic fluid, absent kidneys, and two vessel umbilical cord. MRI showed bilateral PHPV, eye anomalies and microtia, unusual occiput, small cisterna magna, DWM, ventriculomegaly, narrowing of the occiput, bilateral persistent hyaloid vessels with triangular shaped lens, hypertelorism, bilateral microtia, micrognathia, flattened nose, and cleft lip and palate. Chromosome analysis revealed an abnormal karyotype, 47,XX,+22. This is an additional case to the very few reported cases, with different arrays of fetal abnormalities. As with the others, this demonstrates the merit of fetal karyotyping in view of these multiple congenital abnormalities.

Chromosome analysis of uncultured amniocytes by Comparative Genomic Hybridization: A prospective study in early amniocentesis. *C. Vuthiwong¹, N. Aranyakasemsuk¹, C. Tocharoentanaphol², A. Ketupunya¹, S. Muttamara¹.* 1) Obstetrics and Gynecology, Phramongkutklo Hospital, Bangkok, Thailand; 2) Ramathibodi Hospital, Mahidol University.

Objective: To study chromosome analysis by Comparative Genomic Hybridization method (CGH) compare with conventional method.

Study design: Cross-sectional descriptive study

Populations: 32 singleton pregnant women with gestational age between 12 and 15 weeks without medical nor obstetrical complication who have indications for chromosome analysis. The patients and husbands decide to have early amniocentesis after the genetic counseling and informed consent for participation in the study were obtained.

Methods: Complete family pedigree information and ultrasound was performed to identify gestational age, anomalies and site of puncture. Trans abdominal amniocentesis were carried out under ultrasound guidance by using 20 gauge disposable sterile spinal needles. 1 ml. of amniotic fluid was discarded to prevent maternal cell contamination and 12-15 ml. was collected and divided into 2 parts, one part 4-5 ml. was sent for CGH analysis, another part for conventional chromosome analysis. The patients were follow up in 2-3 weeks for the results, at 20 weeks gestation for ultrasound scanning and antenatal care at 28 weeks gestation.

Main outcomes: Conventional karyotypes is a gold standard technique.

Results: Chromosome analysis of 32 pregnant women by CGH technique can detect 17 female karyotypes (53.1%) and 15 male karyotypes (46.9%) which were the same as the conventional technique. No failure rate of both techniques. The mean duration of the CGH technique was 6 days and 14 days for conventional chromosome analysis.

Conclusion: The CGH technique in chromosome analysis of uncultured amniocytes can detect 32 samples which result of the tests were the same as the conventional technique and less time for analysis.

Prenatal Diagnostic Markers for Cornelia de Lange Syndrome. *D. Yaeger*¹, *D. Driscoll*^{1, 2}, *M.J.M. Nowaczyk*³, *L.G. Jackson*^{1, 4}, *I.D. Krantz*¹. 1) Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia; 2) Department of Obstetrics and Gynecology, The Hospital of the University of Pennsylvania, Philadelphia, PA; 3) Departments of Pathology and Molecular Medicine and Paediatrics, McMaster University and Hamilton Health Centre Corporation, Hamilton, Ontario, Canada; 4) Department of Obstetrics and Gynecology, Drexel University School of Medicine, Philadelphia, PA.

Cornelia de Lange syndrome (CdLS) is a dominant genetic disorder characterized by distinct facial features, hirsutism, growth retardation, limb abnormalities, and neurological impairment. Most cases are sporadic. Recurrence risk for families in which neither parent is affected is estimated at 1.5% due to germline mosaicism. *NIPBL* has been identified as the disease gene in approximately 50% of cases. In families where no mutation in *NIPBL* has been found, prenatal screening of future pregnancies involves serial ultrasounds looking for signs of growth retardation, limb abnormalities, or other structural defects associated with CdLS. The maternal serum marker, PAPP-A, has also been demonstrated to be reduced in the first and second trimester of CdLS pregnancies. Recent reports suggest increased nuchal translucency may be a marker in the first trimester. We present two new cases in which sonographic findings suspicious for CdLS were confirmed after delivery. One child was born to unaffected parents who had a previous child with CdLS. In the subsequent pregnancy ultrasound findings at 19 weeks demonstrated severe micrognathia and bilateral hand anomalies. The diagnosis was confirmed after termination. In the second case there was no family history and the fetus was noted to have prenatal findings consisting of IUGR and severe limb reduction defects, however the diagnosis of CdLS was not considered until late in the pregnancy. Twenty previously reported cases of prenatal findings in CdLS were reviewed to determine the spectrum of physical features and gestational age at which they are evident. These cases reveal a pattern of sonographic findings and low maternal serum PAPP-A levels that should raise the suspicion of CdLS during pregnancy.

Program Nr: 2837 from the 2004 ASHG Annual Meeting

Enrichment of fetal cells from maternal blood. *D.T. Yamanishi, P.G. Hujsak, S.F. Snyder, C. Schmitigal, G. Walker, G. Tao, M. Huang, J. Xu.* AVIVA Biosciences Corp, San Diego, CA.

Non-invasive methods to extract fetal cells from maternal peripheral blood for genetic analysis would be a significant advancement for prenatal diagnosis. To address this un-met need, AVIVA Biosciences Corporation (AVIVA) has developed a complete protocol that allows consistent enrichment and recovery of rare fetal cells from maternal peripheral blood. **OBJECTIVE:** To develop a procedure to reproducibly enrich fetal cells from maternal peripheral blood. **DESIGN:** The two-step protocol involves the Fetal Cell Enrichment Kit (FCEK), which utilizes a negative depletion approach to remove maternal cells and platelets, while minimizing the loss of fetal cells. Interphase FISH was performed to detect fetal cells. **RESULTS:** We have optimized our FCEK based on the positive results from a clinical evaluation at Baylor School of Medicine. Our results demonstrate significant improvements in sensitivity, robustness and overall performance. **CONCLUSION:** AVIVA's optimized FCEK is ideally suited for non-invasive enrichment and cytogenetic analysis of fetal cells.

Preconceptional Multivitamin/Folic Acid Use and Rates of Neural Tube Defects: FASTER Study Data. *Y. Kharbutli¹, K. Eddleman¹, R. Zinberg¹, F. Malone², R. Ball³, D. Nyberg³, C. Comstock³, G. Saade³, S. Gross³, L. Dugoff³, S. Craigo³, I. Timor³, S. Carr³, J. Vidaver⁴, M. Dalton², NICHD.* 1) Mount Sinai Medical Center, NY, NY; 2) Columbia University, NY, NY; 3) FASTER Consortium; 4) DM-STAT.

Objective: To examine rates of neural tube defects (NTDs) and preconceptional multivitamin and/or folic acid use from the FASTER trial database generated from nationally recognized perinatal referral centers. **Study Design:** Participants in the first and second trimester evaluation of risk (FASTER) trial were enrolled between 10-14 weeks of pregnancy. Preconceptional multivitamin/folic acid use and duration was documented by interview questionnaire and participants were followed for complete pregnancy outcome. Comparison of NTD rates versus preconceptional multivitamin use was done using Fishers exact test with multivitamin use defined as daily intake at least one month prior to pregnancy. **Results:** Evaluation of multivitamin use among the 37,866 participants revealed that 17,639 used multivitamins while 20,227 did not. Of the 397 diabetic patients, 155 (or 39%) took vitamins while 66 of 96 (68%) patients with a previous NTD pregnancy took vitamins. Multivitamin use among the entire patient population was found to statistically increase with age (P-value <0.0001). Analysis of usage among three age groups, 16-25, 25-35 and 35 year olds, revealed rates of 23.4%, 50.7%, and 59.8% respectively. Therefore, 33 cases (0.09%) of NTD were observed in the FASTER study population. There were 18 NTD cases (0.10%, 95% CI 0.06%-0.16%) among those that had taken vitamins and 15 NTD cases (0.07%, 95% CI 0.04%-0.12%) among those that had not and this difference was not significant. **Conclusions:** The prevalence of NTDs among our FASTER population was 33/ 37,866 (0.09%). Our data show a significant increase in multivitamin use with increasing maternal age. The NTD cases were found among 0.10% of patients that had taken multivitamins and 0.07% of patients that had not. Although this observation does not show a reduction in NTDs among those taking preconceptional multivitamins, this finding may be reflective of methodological limitations.

First PAGE: Seven year experience with screening for birth defects and genetic disorders by primary prenatal care providers. *E.M. Kloza*¹, *S. Ellingwood*¹, *J. Johnson*², *J.E. Haddow*¹. 1) Foundation Blood Research, Scarborough, ME; 2) Office of Institutional Research, University of Southern Maine, Portland, ME.

Purpose: Prenatal care providers (PCPs) are key to the identification of pregnant women at risk of delivering children with birth defects or genetic conditions. Efforts to engage PCPs in genetic risk identification have had mixed success. We report here a well-accepted strategy that has been used successfully since 1996 to identify and manage women at risk while educating PCPs about genetic issues relevant to their practice. Methods: Dubbed *ProgramME*, the approach used a 15 element self-administered Genetic History Questionnaire (GHQ) indexed to a 15 section Office Guide, and was introduced in 1996 by project staff to 212 Maine PCPs. Eighty-five percent of survey respondents used the GHQ with all new patients and gave the approach a rating of 4.4 out of 5. The number of calls for genetic information or referrals was unchanged following implementation of *ProgramME*. However, the percentage of calls initiated by family history increased from 13.2% to 27.4% ($p < 0.01$), and calls associated with a maternal condition rose from 9.9% to 19.5% ($p = 0.03$). Modified materials were introduced in an additional 7 US venues with similar success. In 2002, the March of Dimes funded a revision and update of *ProgramME*. At that time, 48% of Maine PCPs continued to use the 1996 *ProgramME* materials compared to 85% in 1996. Renamed *First PAGE*, the new materials were introduced to all Maine PCPs and mailed to all New Hampshire PCPs as well. Results: Eighty-three percent of respondents to a survey of Maine participants intended to use the approach with all or most new patients, similar to the response in 1996. Seventy-one percent said *First PAGE* made them more confident discussing genetic issues with patients, and 69% said it simplified risk assessment. It helped 60% learn more about genetics, and assisted 74% in addressing risk early in pregnancy. Conclusions: *First PAGE* is a well-accepted, useful, and important tool for engaging community PCP participation in risk identification and management, but must be introduced office by office by committed project staff to be successfully integrated.

Current prenatal genetic counseling practice with regard to sonographic markers for Down syndrome. *E. Carson, L. Cohen, N. Lazebnik, S. Schwartz, C. Curtis, A. Matthews.* Dept Human Genetics, Case Western Reserve University, Cleveland, OH.

Many studies have been published regarding the association between sonographic markers and the risk for Down syndrome (DS) however no clear guidelines exist regarding how to modify patient risk. This study examined variability of genetic counseling practice regarding significance of sonographic markers, methods of risk adjustment utilized, factors influencing methods used and circumstances under which risk adjustment and/or invasive testing are offered. According to 98% (148/151) of responding genetic counselors, level II ultrasound studies are valuable tools for DS risk assessment based on the presence or absence of markers. Variability in reported risk adjustment methods (quantitatively vs. qualitatively) was noted under two circumstances: 1) in the presence of an isolated marker with low specificity and 2) advanced maternal age. Overall, there does not appear to be consistency in how genetic counselors modify risk. Although the literature suggests that certain markers are more specific than others, counselors did not differentiate between them in terms of qualifying risk for DS. For example, 25% did not associate a thickened nuchal fold with an increased risk while 60% would elevate risk based on an isolated echogenic intracardiac focus. Responses indicated that many additional factors influence counselors practices of risk assessment, most significantly the type of center-specific guidelines regarding offering invasive testing for DS. Although 86.6% of respondents were involved in the development of these guidelines, 48% were only somewhat comfortable with them. Additional issues raised which may contribute to a lack of comfort include uncertainty of published evidence, variable skills of ultrasonographers and provoking anxiety for patients. Moreover, while most research has been conducted on high-risk women, results from this study demonstrate that high-risk assessment data is extrapolated onto a low-risk population. A lack of consensus in ultrasound literature regarding the association of markers with DS makes it difficult to establish practice guidelines at this time.

Midtrimester recurrence risk of aneuploidy in women with a previous history of Down syndrome. *T.M. Sellers, A.E. Donnenfeld, K.M. Annable.* Genzyme Genetics, Philadelphia, PA.

PURPOSE: To determine the recurrence risk of aneuploidy at the time of amniocentesis for women who have had a previous pregnancy with Down syndrome. **METHODS:** A retrospective analysis of our database was performed for all amniocentesis specimens submitted with the indication of prior pregnancy with Down syndrome. This includes a prior prenatal diagnosis or liveborn with Down syndrome. The observed frequency of aneuploidy in this group was compared to the expected rate based on maternal age using a case control comparison. **RESULTS:** There were 1567 amniocentesis specimens submitted because of a prior pregnancy with Down syndrome. Forty-one had a subsequent pregnancy diagnosed with aneuploidy at amniocentesis; 28 were trisomy 21, 6 were trisomy 18, 4 were 47,XXY and 3 were 47,XXX. The mean age of the 1567 women evaluated was 35.13 years of age. The expected frequency of aneuploidy at midtrimester based on a maternal age of 35.13 years alone was 7.68 per 1000. The observed frequency of aneuploidy at midtrimester in our population was 26.16 per 1000. A risk ratio of 3.41 was calculated. This was statistically significant ($p < 0.0001$). **CONCLUSION:** There is a significantly increased risk of aneuploidy at midtrimester in patients who have had a previous pregnancy with Down syndrome ($p < 0.0001$). The risk of aneuploidy at midtrimester in women with a history of Down syndrome is 3.41 times higher than expected based on age alone. The current practice of counseling patients that the recurrence risk of Down syndrome is 1% should be re-evaluated.

Minor dysmorphisms associated with yellow fever vaccine: just blinded association? *D.P. Cavalcanti¹, M.A. Salomao¹, J. Lopez-Camelo², M.A. Pessoto³, H. Sato⁴, E.M. Amaral⁵, P. Papaiordanou⁶*. 1) Perinatal Genetics, Dept. Medical Genetics, FCM, UNICAMP, Campinas, SP, Brazil; 2) Instituto Multidisciplinario de Biologia Celular (IMBICE), Buenos Aires, Argentina; 3) Dept. Pediatrics, FCM, UNICAMP, Campinas, SP, Brazil; 4) Health Secretary, S. Paulo State, S. Paulo, SP, Brazil; 5) Dept. Obstetrics, FCM, UNICAMP, Campinas, SP, Brazil; 6) Dept. Medical Clinics, FCM, UNICAMP, Campinas, SP, Brazil.

The Yellow Fever Vaccine (YFV) has been considered one of the safest known vaccines. However, the use of YFV in pregnant women is still not recommended because its effects are unknown. Recently in Brazil, in a governmental campaign against the disease, 67% of the population from the Campinas region at the Southeast of the country received the YFV. A number of women, unaware that they were pregnant, received the YFV during the campaign. We report a clinical investigation on 304 Brazilian children exposed intra-uterus to YFV. These children were submitted to a dysmorphological evaluation within an age range of between 1 month and 1 year. A reference population was obtained from births at the University Maternity of Campinas from the period 1997-1999, immediately before the vaccination campaign. Malformations were compared between 304 cases and 10,691 births from the previously defined period of the vaccine campaign. Exposed and non-exposed babies to YFV were also compared to other clinical and social variables. For this purpose the reference population has included 762 malformed babies and their 762 controls. The only important risk factor more frequently observed in exposed babies to YFV was vaginal bleeding in the first trimester ($p < 0.05$). The major malformation rate found in this study was 3.3%. However, minor dysmorphisms, specially pigmented naevus, were significantly more frequent ($p < 0.001$) than in the reference population. Although bias cannot be ruled out, we suggest that a remote reproductive risk hypothesis should be considered in future studies involving YFV. (Supported by FAPESP - 01/10556-9 and Health Secretary of the Sao Paulo State).

Sonographic findings in fetuses with de novo and inherited balanced chromosome rearrangements; a series of 1,955 cases. *B. Goldin*¹, *M. Shaham*², *A.E. Donnenfeld*¹. 1) Genzyme Genetics, Philadelphia, PA; 2) Genzyme Genetics, Yonkers, NY.

OBJECTIVE: To determine the frequency of sonographically detected fetal malformations among fetuses with de novo and inherited apparently balanced chromosome rearrangements.

METHODS: A review of our database for chromosome rearrangements identified at amniocentesis and CVS was performed. Indications for prenatal diagnosis were tabulated for cases in which there was knowledge of whether the rearrangement was familial or de novo. Sonographic findings were compared between fetuses with de novo and inherited apparently balanced reciprocal and Robertsonian translocations. A chi squared analysis was performed, comparing the two groups.

RESULTS: Out of a total of 3,076 chromosome rearrangements identified, information on whether the rearrangement was inherited or de novo was available for 1,955 pregnancies. Abnormal ultrasound findings were reported in 46 out of 1144 (4%) fetuses with inherited apparently balanced translocations and in 34 out of 335 (10%) fetuses with de novo apparently balanced translocations. The difference in abnormal ultrasound findings between the de novo and inherited groups was statistically significant, ($p < 0.0001$, odds ratio 2.7).

The following ultrasound findings were observed for the de novo apparently balanced translocations: choroid plexus cysts (8), intracardiac focus (4), nuchal thickening (3), a kidney abnormality (3), multiple unspecified anomalies (3), hydrocephalus (2) and one case each of echogenic bowel, clubbed feet, polyhydramnios, polyhydramnios with cleft lip/palate, esophageal atresia, holoprosencephaly with cleft lip/palate, hypoplastic right heart and posterior fossa cyst, "double bubble" sign, skeletal anomalies, gastroschisis and congenital heart disease.

CONCLUSION: A significantly higher rate of abnormal ultrasound findings was observed among fetuses with de novo compared to inherited apparently balanced translocations. This data confirms that fetuses with de novo chromosome rearrangements are at an increased risk for congenital anomalies compared to fetuses with inherited rearrangements.

Detection of sample mixture in DNA during high throughput genotyping of single nucleotide polymorphism sites.

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With increasing use of high throughput platforms of single nucleotide polymorphism (SNP) genotyping, many laboratories currently handle hundreds of DNA samples for genotyping thousands of SNP sites on a routine basis. Consequently, the chance of inadvertent human or machine errors due to contamination or sample mixture has become non-trivial. This research presents a novel bioinformatic tool to detect, monitor, and control genotyping errors caused by contamination/mixture of DNA of different individuals. We developed maximum likelihood methods for detecting DNA mixture based on multilocus genotype profile and the number of (apparently) heterozygous sites in each sample. Likelihood-based inference of the presence of mixture is more reliable by using multilocus genotype profiles, compared to that based on the number of heterozygous loci. However, inference regarding the number of contributors in the mixture is more accurate on the basis of the number of heterozygous loci. Although the reliability of mixture prediction and the precision of the estimate of the number of contributors in the mixture increase with the number of SNP sites typed, mixture detection has an accuracy of over 99.9% even with 100 SNPs scored. When DNA typing is being done on samples of heterogeneous population origin (i.e., when population substructure is an issue), knowledge of the extent of population substructure (e.g., the coefficient of coancestry or gene differentiation) improves the prediction ability of mixture occurrence. The algorithm is also applicable for panels of SNP sites with variable allele frequencies. The analytical method is validated with computer simulations of different scenarios of mixtures (e.g., with varying number of contributors) constructed from actual sets of multilocus SNP genotypes. The routine can be easily integrated in any laboratory information management system (LIMS) as a module for quality control and quality assurance purposes in high throughput SNP studies. (Research supported by NIH grant GM 41399 to RC).

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A methodology for hunting disease genes under linkage peaks via large scale resequencing. *S. Nelson, M. Ogdie, B. Merriman.* University of California at Los Angeles, Departement of Human Genetics, Rm 5506b Gonda, UCLA Medical Ctr, Los Angeles, CA.

A common approach to hunting genes for complex diseases is to first perform a genome-wide linkage scan, and then follow up on the resulting linkage peaks using association tests against a dense set of common SNPs to further narrow the region. However, this strategy could miss genes in which the risk is due mainly to distinct rare variants. To assay for this requires sequencing individuals to identify rare variants and mutations. Recent technological advances in high-density DNA oligonucleotide microarrays allow array-based resequencing of megabases of DNA in a cost-effective and high-throughput manner. We present an experimental strategy, statistical methodology, and power calculation software for deploying this technology to detect disease genes that involve the rare-variant mechanism, in the context of a comprehensive follow-up on all genes under a linkage peak in an IBD sharing genome scan for a complex disease. For example, by resequencing 90 select subjects, our approach has 90% power to detect rare-variant effects that impact as little as 40% of the linked individuals at a peak, under realistic linkage study parameters and a cost compatible with academic research grants.

Identification of positional candidate genes for peripheral neuropathy disorders using *in silico* subtraction. J.

*Qian*¹, *M. Khajavi*², *G. Saifi*², *D. Armstrong*³, *C. Prange*⁴, *N. Katasanis*¹, *J. Lupski*². 1) Institute of Genetic Medicine and Wilmer Eye Institute, Johns Hopkins Univ, Baltimore, MD; 2) Department of Molecular and Human Genetics; 3) Department of Pathology, Baylor College of Medicine, Houston, TX; 4) Lawrence Livermore National Laboratory, The I.M.A.G.E. Consortium, Livermore, CA 94550.

Although substantial progress has been made in the identification of genes responsible for Charcot-Marie-Tooth disease (CMT), including *PMP22* (peripheral myelin protein 22), *MPZ*, *GJB1* (gap junction protein b1), and *EGR2* (early growth response 2) many CMT patients without CMT1A duplication do not have identifiable mutations in these genes suggesting other peripheral nerve specific genes responsible for CMT. In fact, genetic studies reveal 36 loci linked to CMT phenotype with 24 of the genes having been identified to date. Due to the extreme genetic heterogeneity and rarity of some minor loci, positional cloning to identify additional genes for CMT has been difficult. As a powerful resource for identifying molecular components crucial for myelination, we have established peripheral nerve specific cDNA libraries. Utilizing these libraries as a gene pool, we performed *in silico* subtraction against the EST sequence database (dbEST) to predict EST clusters likely representing genes expressed exclusively or preferentially in peripheral nerve. We identified many known CMT genes but also uncharacterized peripheral nerve specific ESTs that map to regions of the genome that are linked to a CMT phenotype. These latter ESTs may be strong candidate genes for CMT1.

Strong evidence of a major gene with Mendelian transmission involved in Familial Mesial Temporal Lobe Epilepsy (FMTLE). *R. Secolin¹, R.G.M. Ferreira³, C.V. Maurer-Morelli¹, N.F. Santos¹, E. Kobayashi², R.B. Marchesini¹, F. Cendes², H. Kriger³, I. Lopes-Cendes¹.* 1) Medical Genetics, University of Campinas, Campinas, Brazil; 2) Department of Neurology, University of Campinas, Campinas, Brazil; 3) Department of Parasitology, University of São Paulo, São Paulo, Brazil.

Recently, we described a large group of families segregating a distinct type of temporal lobe epilepsy with familial recurrence (FMTLE). Initial pedigree analysis pointed to strong genetic predisposition for development of hippocampal atrophy in these families. The objective was to determine the type of inheritance that can most appropriately explain disease recurrence in FMTLE. We studied 29 unrelated pedigrees, divided into 98 nuclear families. A total of 602 family members, including 147 patients were analyzed. Complex segregation analysis was performed using POINTER software. Parameters estimated were: dominance, displacement, allelic frequency, multifactorial heritability and transmission probabilities. Related models were compared by likelihood coefficient tests, whereas non-related models were evaluated by Akaike Information Criteria (AIC). Minor value of AIC indicates most parsimonious data adjust. We rejected absence of genetic effect ($p = 0,000$), absence of a main gene ($p = 0,000$), autosomal recessive inheritance ($p = 0,000$) and co-dominant model ($p = 0,029$). However, absence of multifactorial inheritance ($p = 0,920$) and autosomal dominant inheritance ($p = 0,987$) were not rejected. Mendelian transmission ($p = 0,000$) and absence of Mendelian transmission models ($p = 0,000$) were rejected. However, AIC indicates that Mendelian transmission is more parsimonious (AIC = 401.603) than absence of Mendelian transmission model (AIC = 527,207). This is the first segregation studied performed in FMTLE. Our results strength evidence for genetic predisposition in this disorder. In addition our data provides additional evidence for a major gene presence with Mendelian transmission, which could be involved in development of hippocampal atrophy in these patients. Linkage studies are under way in order to identify the major gene involved in FMTLE.

A simulation Tool for False Positive Gene Replication in case-control Studies. *C. Stalens, C. Aucan, I. Elias-Billon, A. Guilbot, D. Cohen, H. Tanaka, F. Macciardi.* Serono Genetics Institute, RN7, Evry, France.

Recent improvements in high throughput genotyping technologies, allow to carry out genetic studies on several tens of thousands SNPs. Considering a classical case-control study (allelic and genotypic associations) on 100 000 SNPs, 200 000 statistical tests are performed. This large number of tests leads to a large number of false positive results as well (in this case, 10 000 false positive SNPs with a type I error = 0.05). Traditional methods to correct for multiple testing in these cases are no longer easy to apply while, as a complementary strategy, replication studies have been claimed to be useful to deal with such a multiple testing problem. Using a simulation study, we estimated the number of false positive findings before and after replication of a case-control study. In this simulation, genotypes were randomly generated without distinction between cases and controls, i.e. assuming a priori no true positive findings. We then defined a gene as a set of X consecutive SNPs and generated Y replicates of a case-control population composed by an equal Z number of cases and controls. We evaluated a complete replication design, i.e. a study where the entire set of around 30000 genes has been simulated in 2 different samples. We considered a gene "positive", i.e. associated to a hypothetical disorder, if at least two of these X SNPs presented a "significant allelic or genotypic association", significance threshold p for the p-value being variable. According to the chosen significance threshold for association, we thus tested various parameter combinations to estimate the number of false positive results remaining after replication. We investigated the effects introduced by different minor allele frequency threshold, by different number of SNPs within a gene and by varying the number of cases and controls. With the classical used significance threshold ($p=0.05$), we roughly observed a replicated false positive rate over the total number of genes from 0.4 % to 0.95 %. This rate was considerably reduced by decreasing the significance threshold for type I error at 1 %.

Longitudinal Models for Interactions between Gene and Time Varying Environments in Qualitative Traits. *M. Sun*¹, *M. Xiong*². 1) Dept Business, Univ Texas, San Antonio, San Antonio, TX; 2) Human Genetics Center, University of Texas at Houston.

Diseases are largely caused by dynamical interaction between the genes and environments. Such interactions will lead the changes of the phenotypes over time. A trait that varies as a function of a continuous variable such as age or time is referred to as a function-valued trait. Longitudinal data that are collected on a certain number of occasions for each individual can be used to study function-valued traits. Genetic studies of function-valued traits are a natural extension of classical genetics. Genetic analysis of function-valued traits will provide a powerful tool for identifying complex genetic structure of common diseases and discovering strategies for prevention of diseases. Genetic analysis of function-valued traits should be an integral part of genetic theory. However, genetic models of function-valued traits have not been well developed. In this report, we propose a general framework for genetic analysis of function-valued qualitative traits in which the relationship between the qualitative trait and DNA variation is modeled by a generalized mixed linear model. The mean and genetic effect functions are modeled as fixed effects. Correlation structure of repeat measurements is modeled as a random effect. The REML method is used to estimate the mean and genetic effect functions as well as variance components in the mixed linear model. We propose several hypotheses and test statistics to test association of candidate locus with the qualitative diseases. The methods are applied to simulation data to evaluate their performance and to illustrate their applications.

A statistical clustering method for classifying the Invader assay genotyping data. *S. Takitoh¹, S. Fujii¹, Y. Mase², N. Kamatani^{3,6}, T. Yamazaki³, Y. Ohnishi⁴, Y. Nakamura⁵, M. Yanagisawa¹.* 1) Department of Computer Science, Waseda University, Shinjuku, Tokyo, Japan; 2) NTT DATA Corporation, Tokyo, Japan; 3) Laboratory for Statistical Analysis, Research Group for Personalized Medicine, SNP Research Center, RIKEN, Tokyo, Japan; 4) Laboratory for SNP Analysis, Research Group for Personalized Medicine, SNP Research Center, RIKEN, Tokyo, Japan; 5) Research Group for Personalized Medicine, SNP Research Center, RIKEN, Tokyo, Japan; 6) Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan.

The Invader assay is a method for identifying SNP genotypes. Information on the genotype for each SNP is given as two fluorescent intensity values. The genotype is classified on the basis of these two-dimensional points. The classification results by the existing methods have not been necessarily identical to those by the experts. Eventually, the exact results have required large time. We propose a new statistical clustering algorithm, which is implemented in Genocluster, on the basis of maximum likelihood estimation by the Markov Chain Monte Carlo (MCMC). MCMC depends on an initial value. In the Genocluster, the nearest neighbor is applied to the initialization. The Genocluster can be effective even if the number of clusters is uncertain and the number of points in clusters is few. Genocluster is compared with the existing method by using large-scale real data. The results demonstrate high accuracy of the algorithm. Genocluster enabled us to automatically classify the Invader assay SNP genotypes more accurately than the existing methods.

Program Nr: 2851 from the 2004 ASHG Annual Meeting

Dynamical Models for Quantitative Genetics. *M. Xiong, J. Zhao, E. Boerwinkle.* Human Genetics, University of Texas Health Sci, Houston, TX.

Most phenotypic variations, including those involved in complex diseases and differences in drug response, are generated by integrated actions of multiple genetic and environmental factors, through dynamic, epigenetic, and regulatory mechanisms. A complex trait should be taken as a complex biological system that experiences dynamical changes. State-space equations in engineering are a powerful tool for modeling dynamics of the complex systems. To apply state-space approach to genetic studies of complex traits, we first formulate the quantitative genetics as a control problem in which the varying genetic effects over time are modeled as state variables, the environments are modeled as inputs, the observed quantitative phenotype is modeled as an output variable, and the genotypes are related to phenotypes via observation equation in the model. The statistical methods for estimation of the dynamically changing genetic effects have been developed. A statistic for testing the presence of the trait locus is proposed. Both analytic and simulation methods for power calculation have also been developed. Simulations of the steady-state and transient response of the trait to environmental changes have been performed. The proposed dynamical model was applied to the Coronary Heart Disease.

Program Nr: 2852 from the 2004 ASHG Annual Meeting

Global Test for Genome-Wide Association Studies. *J.Y. Zhao, M.M. Xiong.* Human Genetics Ctr, Univ Texas, Houston, Houston, TX.

With the great progresses in HapMap Project, hundreds of thousands or even millions SNPs markers have been or will be available for genome-wide association studies of complex diseases. This opens the new avenue for dissecting the genetics of complex diseases, however, this also presents great challenge. The questions arise as to how to simultaneously utilize multiple markers and how to deal with multiple testing problems. A new statistical test for genome-wide association studies has been developed, which allows (1) using any number of SNPs markers, (2) using haplotypes and (3) selecting optimal set of markers, and does not have multiple testing problem. The degree of freedom of the developed test statistic is very low even for a large number of SNPs markers used. Both theoretical analysis and simulation study show that the proposed global test for genome-wide association studies has higher power than the classical 2 test and the generalized T2 test. The proposed test was applied to genome-wide association studies of the cardiovascular disease. The results are promising.

Relationship between bone mineral density and cardiovascular disease differs by sex. *J.R. Shaffer¹, C.M.*

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Previous studies have noted an association between osteoporosis or bone mineral density (BMD) and cardiovascular disease (CVD) in women, but little is known about the relationship in men, and the underlying mechanisms are unclear. We used maximum likelihood methods, conditioned on the pedigree structure, to estimate correlations between BMD, IMT (intimal-medial thickness of the carotid artery a measure of CVD), and lipid levels, while accounting for potential covariates, including age, body mass index, and menopausal status. These traits are moderately to highly heritable in men and women. Data were available on 527 women and 327 men (average age = 43 years) from 34 families recruited as part of the San Antonio Family Osteoporosis Study (a population-based study). As expected, spine BMD in women decreased with increasing IMT ($p < 0.001$). In addition, radius and hip BMD decreased with increasing IMT in older women, but increased with increasing IMT in young women ($p < 0.007$ for both traits). In men, we observed no relationship between IMT and spine or hip BMD, but the age by IMT interaction effect on radius BMD was similar to that in women ($p < 0.001$). Further investigation revealed that in women, low-density lipoprotein cholesterol (LDLC) levels were strongly negatively-correlated ($p < 0.01$) with spine and hip BMD, and accounted for much of the relationship between IMT and BMD. There was no relationship between LDLC and BMD at any site in men or between LDLC and radius BMD in women. We conclude (1) that the effects of common etiologic factors affecting BMD and CVD are mediated differently in men and women, at different bone sites, and at different ages, and (2) one or more pathways that influence hyperlipidemia may also affect spine and hip BMD in women.

Genetic and Environmental Determinants of Osteoporosis Phenotypes in Multigenerational Afro-Caribbean Pedigrees: The Tobago Family Health Study. *J.M. Zmuda¹, X.J. Wang¹, M. Kenney¹, V. Wheeler², A.L. Patrick², J.A. Cauley¹, C.H. Bunker¹, C.M. Kamerer¹.* 1) Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 2) Tobago Health Studies Office, Scarborough, Tobago.

Osteoporosis (OP) and its associated fractures is a frequent and major cause of disability and medical costs worldwide. Although the prevalence of OP is lower in African than European populations, the incidence increases considerably with age so that the absolute number of affected African individuals is expected to increase dramatically during the next half century, due to increases in life expectancy. In contrast to our understanding of bone health in European populations, there is a paucity of information about bone health in African populations. We have begun an initiative to disentangle the genetic and environmental contributions to the bone strength phenotype in multigenerational families of African origin. Dual-energy X-ray absorptiometry (DXA) is used to assess integral bone density (BMD) and cross-sectional area (CSA) at several skeletal sites. Quantitative computed tomography measures of trabecular and cortical volumetric BMD and architecture are also obtained. We present preliminary results on age and gender effects and residual heritability (h^2_{rSE}) of DXA phenotypes in the first 190 individuals (43% male) aged 18 to 86 yrs (mean, 43 yr) who are members of 7 large, extended pedigrees. Age and gender explained 13-22% of the variance in spine, femoral neck and whole body BMD and femoral neck CSA. In contrast, there was highly significant evidence ($P < 0.001$) for a genetic contribution to these traits. Residual h^2 of BMD phenotypes was: 0.420.13, 0.360.14, 0.400.14, respectively. Femoral neck CSA, an indicator of bone size, showed the highest residual heritability (0.550.17). These results establish a strong familial resemblance in integral BMD and bone size traits in pedigrees of African descent. These osteoporosis traits should be informative for genome-wide linkage studies to identify the loci contributing to the bone strength phenotype. Such analyses should greatly advance our understanding of the genetic susceptibility to osteoporosis and yield fundamental insight on bone biology.

Aneurysmal Subarachnoid Hemorrhage: Associations with variants in Apolipoprotein E and Elastin genes. R. Kaushal¹, D. Woo², P. Pal¹, M. Haverbusch², C. Moomaw², B. Kissela², Q. Zhang¹, H. Xi¹, L. Sauerbeck², G. Sun¹, R. Chakraborty¹, J. Broderick², R. DeKa¹. 1) Dept Environmental Health, Univ Cincinnati, Cincinnati, OH; 2) Dept Neurology, Univ Cincinnati, Cincinnati, OH.

Subarachnoid Hemorrhage (SAH) caused by ruptured intracranial aneurysms has an annual incidence of 6-10 per 100,000 in U.S. and a 30-day mortality rate exceeding 40%. There is strong evidence that both genetic and environmental risk factors interact in its etiology. Apolipoprotein E (*ApoE*) and Elastin (*ELN*) genes are important candidates in the pathogenesis of SAH. *ApoE* variants have been associated with lipid disorders, cardiovascular and cerebrovascular diseases. Polymorphisms in *ELN* have inconsistently been implicated in cerebral aneurysms. However, these associations remain inconclusive. We have performed a case-control (age, race, gender matched) association study of aneurysmal SAH using 12 single nucleotide polymorphisms (SNPs) located in upstream and the coding region of the *ApoE*, and 9 SNPs (8 intronic and 1 coding) in the *ELN* in a sample of 381 individuals of Caucasian and African-American ancestry (133 cases, 248 controls). Genotype frequencies were in conformity with HWE proportions at all markers. Haplotype analysis using the PHASE program revealed significant association with one major haplotype in *ApoE* in Caucasians ($p = 0.008$). When the upstream and coding haplotypes were separately inferred, we found significant association with a major upstream haplotype in both Caucasians ($p = 0.04$) and African Americans ($p = 0.02$). We did not find any association of the *ApoE* 2/3/4 variants (either at allelic or genotypic level) with aneurysmal SAH. However, haplotype data from the *ApoE* upstream region suggests that *ApoE* promoter polymorphisms significantly contribute to the risk of aneurysmal SAH in both African Americans and Caucasians. We did not find any association of *ELN* variants with aneurysmal SAH, as was previously reported by Onda et al. (*Am J Hum Genet* 2001, 69:804-819), at both allelic or haplotype level in either African Americans or Caucasians. Our results indicate that *ApoE* plays an important role in the pathogenesis of SAH. This study was supported by NIH grant NS36695.

BDNF and its specific receptor, tyrosine kinase B (NTRK2), are associated to eating disorders and related psychopathological traits. *M. Ribases¹, M. Gratacos¹, JM. Mercader¹, A. Badia², L. Jimenez², R. Solano², F. Fernandez-Aranda², X. Estivill^{1, 3}.* 1) Center for Genomic Regulation; 2) Hospital Princeps Espanya; 3) Pompeu Fabra University, Barcelona, Catalonia.

Genetic studies and animal models suggest that brain-derived neurotrophic factor (BDNF) has an essential role in eating behavior, and that alterations of this neurotrophic system could participate in eating disorders (ED). BDNF and its specific receptor tyrosine kinase B (NTRK2) knockout mice develop obesity and hyperphagia. We have reported a strong association of the -270C/T and Val66Met BDNF SNPs with anorexia (AN) and bulimia (BN) in 6 European populations. We present here association of ED with the NTRK2 receptor. The NTRK2 gene was screened in 91 ED patients and 8 SNPs and 6 single mutations were identified. Of these, six SNPs, -69G>C, IVS2+40T>C, IVS13+40G>A, IVS17-125T>C, IVS18+13G>A and 2784-2785insC, showed a minor allele frequency higher than 10% and were used to carry out a case-control study in a sample of 168 ED patients and 121 unrelated controls. We have found that the -69C allele of the -69C>G SNP is associated to purging AN (ANP; $p=0.016$) and minimum body mass index ($p=0.001$), and that the IVS13+40G>A SNP has an effect on BN ($p=0.03$). Considering the linkage disequilibrium pattern, we analyze the -69G>C, IVS13+40G>A and 2784-2785insC SNPs to estimate haplotypes and observed a positive association of the CAC haplotype with ANP ($p=0.006$). Moreover, patients with BN showed a reduced frequency of the GA- haplotype ($p=0.034$). We also analyzed the possible involvement of the NTRK2 SNPs in different psychopathological traits associated to ED. Thus, 84 ED patients completed the Symptom Checklist 90-revised questionnaire and were genotyped for the NTRK2 variants. We observed that those patients carrying the IVS17-125T allele of the IVS17-125T>C SNP showed increased symptomatology for some subscales that include somatization ($p=0.005$), obsessive-compulsiveness ($p=0.006$) and anxiety ($p=0.04$). These results suggest that not only BDNF but also its receptor NTRK2 could participate in ED, not only through its effect on eating behavior but also on related psychopathological traits..

Segregation Analysis of Thyroid Cancer using the Swedish Family Cancer Database. *A. Ding¹, A. Antoniou¹, K. Czene², K. Hemminki^{2,3}, D.F. Easton¹.* 1) Cancer Research U.K. Genetic Epidemiology Unit, University of Cambridge, Strangeways Research Laboratory, Cambridge, United Kingdom; 2) Karolinska Institute, Stockholm; 3) German Cancer Research Center (DKFZ), Heidelberg.

The Swedish Family Cancer Database is constructed by record linkage of a population-based family register with the Swedish Cancer Registry, and provides a basis for large scale pedigree analysis using population-based families. We have implemented routines in MENDEL to perform segregation analysis on these data. Survival analysis models, incorporating age- and sex-specific hazard rates and adapted to constrain overall incidence rates to agree with population rates, are fitted (1). A generalised estimating equation approach is used to account for multiple ascertainment of probands.

We have applied this approach to thyroid cancer. A total of 7465 pedigrees containing first and second degree relatives of thyroid cancer probands, 201 of which had a family history for the disease, were analysed. To date, the best fitting model postulates a rare autosomal dominant locus ($q=0.00004$). Under this model, cumulative risks to female (male) gene carriers were 63.6% (34.8%) and 87.6% (38.3%) by ages 50 and 70 years, respectively. The higher risk in female carriers mirrors the difference in rates in the general population. These models can provide the basis for counselling of relatives of thyroid cancer patients.

(1) Antoniou et al (2002) Brit J Cancer 86:76-83

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Evidence Against Autosomal Dominant Inheritance in Familial Beckwith-Wiedemann Syndrome. M.F.

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Beckwith-Wiedemann syndrome (BWS) is a congenital disorder with epigenetic and genetic mutations on 11p15. A small proportion of cases are familial with an unknown pattern of inheritance, although autosomal dominant inheritance has been proposed. We tested the hypothesis that familial BWS occurs as a result of dominant inheritance. Segregation analysis based on a revised general model in which two parameters were not estimated was performed for a discrete trait in 291 families from The BWS Registry ascertained for an affected child (see Table below). Affected status was assigned using two of five major features, macroglossia, macrosomia, hypoglycemia at birth, abdominal wall defects, and ear defects. Models of inheritance were evaluated with the Likelihood Ratio Test ($-2 \ln L$) and Akaike's Information Criteria (AIC). Sporadic and environmental models were rejected. Of the Mendelian models, additive and recessive models were equally likely and were the best fit to the data while the dominant model was rejected. We have provided preliminary evidence that dominant transmission is not the most likely mode of inheritance but is still possible in a subgroup of families. The inheritance pattern of Familial BWS is likely heterogenous.

Segregation Analysis in 291 Families Ascertained for a Child with BWS

Model	Sporadic	Recessive	Additive	Dominant	Environmental	General
-2 ln L	4129	2703	2703	2711	2711	2703
P value	<0.001	1	1	0.003	0.005	-
AIC	4129	2709	2709	2717	2719	2713

The effect of germline p53 mutations in segregation analysis of cancer in families with childhood soft tissue sarcoma. *C.C. Wu¹, S. Shete¹, C.I. Amos¹, L.C. Strong²*. 1) Dept Epidemiology; 2) Section Clinical Cancer Genetics, Dept Molecular Genetics, Univ Texas MD Anderson CA Ctr, Houston, TX.

The study of familial cancer incidence was performed in 159 families ascertained through childhood soft tissue sarcoma who were treated in M. D. Anderson Cancer Center. Our previous research showed that a highly significant excess of familial cancer was observed in both the first-degree relatives of the patients and the relatives of the patients with second malignant neoplasms. The tumor types occurring in close relatives were also observed as second malignant neoplasms in the patients. Germline p53 mutations have been identified in 50%-70% families with Li-Fraumeni syndrome. We have performed p53 mutation analysis in 107 of these 159 kindreds, and identified a total of 63 carriers in 7 kindreds. To test hypothetical models of disease transmission, we include confirmed invasive cancers (excluding nonmelanoma skin cancer and in situ carcinoma) as a combined phenotype. Assuming that the affection of cancer follows a logistic distribution, a segregation analysis was performed to evaluate sex effects, a generation cohort covariate, and p53 mutation covariate. Incorporating p53 as a covariate significantly increases the likelihood values for all genetic models. We found the odds of the affected individual carrying p53 mutation to be significantly high. It was over five fold higher on the logit scale for all genetic models. In contrast, we also performed additional segregation analysis in four large kindreds without p53 mutations.

Linkage Disequilibrium Structure and Selection of Tagging-SNPs are Insensitive to Phenotype: CETP and Coronary Artery Disease. *B.D. Horne^{1,2}, J.F. Carlquist^{1,3}, J.B. Muhlestein^{1,3}, J.L. Anderson^{1,3}, N.J. Camp^{1,2}.* 1) Cardiovascular Department, LDS Hospital, Salt Lake City, UT; 2) Genetic Epidemiology, University of Utah, SLC, UT; 3) Cardiology Division, University of Utah, SLC, UT.

For complex diseases such as coronary artery disease (CAD), evaluation of all common SNPs (>5%) and selection of informative subsets is recognized as best practice for association analyses. We introduced a principal component analysis (PCA) method for identification of linkage disequilibrium (LD) groups and tagging SNPs (tSNPs). However, it is unknown whether phenotype influences LD structure or tSNP selection. The current study applied the PCA method to 35 SNPs in the cholesteryl ester transfer protein (CETP) gene. Nineteen common SNPs were identified by scanning the promoter, exons, and 3' UTR in 50 unrelated individuals. A further 16 SNPs from the literature were used, with the total 35 SNPs genotyped in 529 unrelated individuals, including 339 population healthy controls (mean age 59.13yrs, 67% female) with no history of CAD and 190 non-diabetic, non-smoking patients (53.8yrs, 55% female) with significant, angiographically-determined CAD (2 lesions of 70% stenosis). In controls, 7 LD groups were identified and 11 tSNPs selected, accounting for 93.4% of the genetic variation. The LD groups were overlapping and non-contiguous, conditions violating simple 'block' methods. In the CAD patients, 8 LD groups were found, 7 essentially the same as in controls and the 8th defined by a single SNP occurring at higher frequency in CAD patients; 12 CAD tSNPs were selected (the 12th being the sole group 8 SNP), accounting for 93.9% of the variation. Three CAD tSNPs differed from the control tSNPs, but were in high LD with 3 control tSNPs and could be substituted with a negligible reduction in variance explained. This study of the CETP gene demonstrates a high correspondence of LD structure and tSNPs among two populations with divergent phenotype profiles, suggesting it may be reasonable to use varied population compositions when identifying tSNPs. It also highlights the importance for tSNP selection of methods such as PCA that allow for the increased complexity of LD structure in intragenic regions.

The Impact of Pedigree Structure on Heritability Estimates for Pulse Pressure in Three Studies. *F-C. Hsu¹, D.J. Zaccaro¹, L. Lange¹, D.K. Arnett², C.D. Langefeld¹, L.E. Wagenknecht¹, D.M. Herrington³, S.R. Beck¹, B.I. Freedman³, D.W. Bowden^{3,4,5}, S.S. Rich¹.* 1) Public Health Sciences, Wake Forest Univ, Winston-Salem, NC; 2) Epidemiology, Univ of Minnesota, Minneapolis, MN; 3) Internal Medicine, Wake Forest Univ, Winston-Salem, NC; 4) Center for Human Genomics, Wake Forest Univ, Winston-Salem, NC; 5) Biochemistry, Wake Forest Univ, Winston-Salem, NC.

Pulse pressure (PP), the difference between systolic and diastolic blood pressure, is a measure of large artery stiffness and has been shown to be an important predictor of cardiovascular morbidity and mortality. The aims of the present study were to investigate the heritability of PP in three studies, the Diabetes Heart Study (DHS), the Insulin Resistance Atherosclerosis Family Study (IRAS FS), and the NHLBI Family Heart Study (FHS), to estimate the residual heritability after inclusion of a common set of covariates, and to investigate the impact of pedigree structure on estimating heritability. DHS is primarily a sibling pair nuclear family study design, while both IRAS FS and FHS have large pedigrees. Heritability estimates of log-transformed PP were obtained using variance component models. After adjusting for age, gender, ethnicity/center, height, diabetes status, and mean arterial pressure, heritability estimates of PP were 0.400.08, 0.220.05, and 0.190.03 in DHS, IRAS FS, and FHS, respectively. The heritability estimate from DHS was significantly different from both IRAS FS and FHS. A random resampling technique (bootstrap) was used to explore the heritability in the IRAS FS and FHS data when these pedigrees were trimmed to mimic the DHS pedigree structure. Heritability estimates and their 95% confidence intervals were computed. The bootstrap method yielded PP heritability estimates of 0.37, 0.34, and 0.27 in DHS, IRAS FS, and FHS, respectively. There was no significant difference among the estimates from the three studies based on this method. These data illustrate the influence of pedigree structure can have on estimating heritability. Thoughtful comparisons of heritability estimates must consider study design factors such as pedigree structure.

A collection of nucleotide variations in SCN5A, a major arrhythmogenic gene, among the Japanese. *J. Mashiba, G. Koike, H. Kamiunten, M. Ikeda, A. Chishaki, K. Sunagawa.* Cardiovascular medicine, Kyushu University, Fukuoka, Fukuoka, Japan.

SCN5A (sodium channel, voltage-gated, type V, alpha polypeptide) is responsible for the initial upstroke of the action potential, and mutations of this gene causes a wide variety of arrhythmias, such as long QT syndrome type 3 (LQT3), Brugada syndrome (BrS), idiopathic ventricular fibrillation and other conduction disorders. In a last decade, it has been reported that various mutations and polymorphisms of SCN5A are linked to these arrhythmias, and that there may be ethnic variations of SCN5A resulting in these arrhythmias. Then, we screened SCN5A of two clinically diagnosed unrelated LQT3 patients, four unrelated BrS patients and twenty-four healthy subjects in the Japanese. Regarding our LQT3 patients, the ST-T wave patterns of their ECG were either biphasic T wave pattern or asymmetrical peaked T wave pattern. We prepared genomic DNA from blood with standard procedure after obtaining informed consent from each participant. All exons covering entire coding region and exon-intron boundaries of SCN5A were amplified by PCR followed by direct sequencing. We identified 16 variations in its coding region; 14 nucleotide variations in its coding region compared to NCBI sequence database, 5 ones compared to DDBJ sequence database. Three nucleotide variations were identical for both databases. Among these variations, two variations were novel. We also identified one novel variation at the acceptor site of exon 13 compared to NCBI database, that was found in one BrS patient. In coding regions, there were no nucleotide variations that result in amino acid substitution among our LQT3 and BrS patients, nor healthy subjects in this study. However, one variation at the acceptor site of exon 13 that was found in one BrS patient could alter its cDNA structure. Therefore, further analysis to elucidate whether this variation affects the function of SCN5A is now undergoing. In addition, our study strongly demonstrated that it is important to characterize nucleotide sequence of any disease genes, since there are ethnic differences of the gene sequence.

Genetic association study of QT interval duration in the Framingham Heart Study. *C. Newton-Cheh^{1,2,3,5}, S. Musone¹, J. Drake^{1,4}, A. Lochner¹, S. Kathiresan^{1,2,3,5}, R.S. Vasan^{2,6}, D. Levy^{2,5}, E.J. Benjamin^{2,6}, C.J. O'Donnell^{1,2,3,5}, J.N. Hirschhorn^{1,4,5}.* 1) Medical & Population Genetics, Broad Institute, Cambridge, MA; 2) NHLBI's Framingham Heart Study, Framingham, MA; 3) Massachusetts General Hospital, Boston, MA; 4) Children's Hospital of Boston, MA; 5) Harvard Medical School, Boston, MA; 6) Boston University, Boston, MA.

Electrocardiographic QT interval prolongation, a marker of delayed myocardial repolarization, is associated with increased risk of sudden cardiac death (SCD) and coronary heart disease in the general population. The congenital Long QT Syndrome (LQTS) is a rare, Mendelian syndrome characterized by syncope and SCD. QT interval duration across the normal range, adjusted for heart rate and sex, is a heritable phenotype ($h^2=0.35$). To elucidate the genetic determinants of this complex phenotype, we are carrying out candidate gene association studies. We are developing high-resolution linkage-disequilibrium (LD) maps of 6 genes previously associated with the LQTS (KCNQ1, KCNH2, SCN5A, ANK2, KCNE1 and KCNE2), encoding potassium and sodium channels and an anchoring protein. By genotyping public database single nucleotide polymorphisms (SNPs) in 93 Caucasian CEPH individuals from 12 multi-generational pedigrees, we are identifying a set of polymorphic SNPs across a combined genomic span of 875kb. A parsimonious set of tagging SNPs captures the majority of common variation in these genes. We are also identifying missense variants by re-sequencing the 108 exons of the 6 genes in a multi-ethnic panel of 40 unrelated individuals. We are testing the tagging and missense SNPs in 1811 unrelated Framingham Heart Study (FHS) participants for association with continuous QT interval variation. To date we have genotyped 1000 SNPs in the CEPH pedigrees (600 with minor allele frequency >5%) and have completed genotyping of tagging SNPs for KCNH2 and KCNE1 in FHS. The comprehensive SNP-based haplotype map of these genes and the planned genotype-phenotype association studies will offer insights into the determinants of myocardial repolarization in a large, community-based Caucasian sample and will generate candidates to test for association with arrhythmias and SCD.

Association of 12/15 Lipoxygenase gene polymorphisms with CRP levels: The Diabetes Heart Study. *S.S. Rich¹, K.P. Burdon¹, C.C. Hedrick², S.R. Beck¹, C.D. Langefeld¹, B.I. Freedman¹, J.J. Carr¹, D.M. Herrington¹, D.W. Bowden¹.* 1) Dept Public Health Sciences, Wake Forest Univ Sch Medicine, Winston-Salem, NC; 2) Cardiovascular Research Center, University of Virginia Health Sciences, Charlottesville, VA.

Atherosclerosis is a major complication of diabetes, involving arterial inflammation, recruitment and adherence of monocytes, macrophages and dendritic cells to the vessel walls, and lipid-laden plaque formation. Regulation of the 12/15 lipoxygenase (12/15 LO) pathway is an important mediator of inflammation and vascular change in the mouse *db/db* model of diabetes. In humans, 12/15 LO consists of two genes (ALOX12, ALOX15) on chromosome 17p13.2 that have recently been sequenced. The role of variants in the ALOX12 and ALOX15 genes was determined on a recognized marker of chronic inflammation, CRP, in the Diabetes Heart Study (DHS), a study of markers of atherosclerosis in families with two or more diabetic siblings with well-preserved renal function. ALOX12 was surveyed for 5 SNPs [1158 (promoter), 2987 (intron 2), 5431 (intron 6), 7605 (exon 8 [synonymous]), 17947 (3)] and ALOX15 for 3 SNPs [1054 (promoter), 6686 (intron 7), 11256 (intron 12)], chosen for high minor allele frequency and coverage of existing haplotype blocks. SNPs frequencies were consistent with Hardy-Weinberg proportions. In the DHS population, two haplotype blocks were observed for ALOX12 (1158-2987-5431-7605; 17947) and ALOX15 (1054; 6686-11256). Association analyses used GEE1 modeling and adjusted for age, gender, ethnicity, diabetic status, smoking and lipid medication use. There was consistent evidence for association of CRP with SNPs in the first ALOX12 haplotype block (1158-2987-5431-7605) spanning the promoter through exon 8 ($P < 0.05$). This effect was strongest in Caucasians, and particularly in Caucasian diabetics, with the SNP most strongly associated under a recessive model. Evidence for association was also significant for the ALOX15 promoter SNP (1054; $P < 0.05$). These results suggest that genetic variants in human lipoxygenase genes are a significant contributor to CRP levels, thereby serving as modulators of atherosclerosis.

Heritability of pulse wave velocity and carotid intima-media thickness in an extended pedigree from an isolated population. *F.A. Sayed-Tabatabaei¹, M.J. van Rijn¹, Y. Aulchenko¹, B.A. Oostra², J.C.M. Witteman¹, C.M. van Duijn¹.*
1) Department of Epidemiology & Biostatistics; 2) Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, The Netherlands.

Pulse Wave Velocity (PWV) and carotid Intima Media Thickness (IMT) represent arterial wall stiffness and thickness respectively. Both traits are associated with cardiovascular diseases but little is known of their heritability. We studied the heritability of PWV and IMT in an extended pedigree from an isolated population in the Netherlands. PWV was measured in 807 and common carotid IMT in 924 individuals belonging to a single pedigree. For 801 individuals both measurements were available. Natural logarithm transformations of both traits were used in the SOLAR software to estimate heritabilities.

Covariates used in the complete multivariable-adjusted model of PWV were age, sex, mean arterial pressure, LDL-cholesterol, fasting glucose and heart rate. For IMT the covariates were age, sex, BMI, systolic and diastolic blood pressure, LDL- and HDL-cholesterol, fasting glucose, smoking and heart rate. The age and sex-adjusted and multivariable-adjusted heritability (standard error) for PWV were 0.36 (0.09) and 0.33 (0.10). For IMT these statistics were 0.41 (0.07) and 0.37 (0.08), respectively (p for all estimates <0.001). The proportion of PWV variance due to all covariates was 0.46 and for IMT was 0.66. The two traits shared between 0.30 and 0.36 of their genetic component (in multivariable-adjusted and age and sex-adjusted models respectively).

To our knowledge, this is the first report on heritability of PWV. Our findings suggest that a substantial part of variance in vessel wall stiffness and thickness are explained by genetic factors.

A multilocus model for essential hypertension susceptibility in a Japanese cohort. *S.M. Williams¹, H. Sanada², M.D. Ritchie¹, J. Yatabe², S. Midorikawa², S. Hashimoto², T. Watanabe², M. Sasaki², P.A. Jose³, R.A. Felder⁴.* 1) Center Hum Genet Research, Vanderbilt Univ Medical Ctr, Nashville, TN; 2) Third Department of Internal Medicine, Fukushima Medical University, School of Medicine, Fukushima, Japan; 3) Georgetown University Medical Center, Washington, DC; 4) University of Virginia Health Sciences Center, Charlottesville, VA.

Hypertension is a complex disease with multiple putative genetic risk factors. In order to determine the genetic architecture of essential hypertension, we evaluated 12 polymorphisms in five candidate loci in a cohort of 447 Japanese subjects (189 hypertensive subjects and 258 normotensive controls) for association to hypertension. One of the loci, G-protein coupled receptor kinase 4 (GRK4), exhibited significant allelic associations at three studied sites (R65L, A142V, A486V) in all subjects with significant genotype associations at the first two sites and a borderline value ($p=0.054$) at the third. In males there was a significant allelic association with A142V and in females there were significant allelic and genotypic associations at R65L and A142V. In females there was also a significant genotypic association for Cyp11B2 (C-344T) but no allelic association. These results indicate a complex set of genetic factors that interact with gender to predispose to hypertension. To evaluate the data we assessed all polymorphic sites simultaneously and determined the single best genetic model. This model includes GRK4 sites R65L, A486V, the insertion deletion in ACE intron 16 and the Cyp11B2 variant. This model predicts hypertension status correctly 61% of the time and is highly significant ($p=0.017$). We determined the odds ratio (OR) for the high risk genotypes from this model to be 5.05 (3.29-7.74, 95% CI, $p<0.01$). This OR is much larger than most of those for other genetic risk factors for hypertension, suggesting that a multilocus model such as the one we found is more informative than any single locus analysis in predicting hypertension status.

Multivariate genetic analysis of chronic pelvic pain and associated phenotypes. *K.T. Zondervan¹, L.R. Cardon¹, S.H. Kennedy², N.G. Martin³, S.A. Treloar³.* 1) Wellcome Trust Centre for Human Genetics, University of Oxford, United Kingdom; 2) Nuffield Dept. of Obstetrics & Gynaecology, University of Oxford, United Kingdom; 3) Queensland Institute of Medical Research, Brisbane, Australia.

Chronic pelvic pain (CPP), a condition of uncertain aetiology involving long-standing pain localising to the lower abdomen and pelvis, is very common in women of reproductive age. It is difficult to diagnose and treat, and causes a substantial burden of functional disability. CPP has been suggested as a phenotype worth pursuing per se for gene discovery studies. Although heritability estimates have been published for some conditions potentially underlying pelvic pain, the heritability of CPP itself has never been investigated. We analysed data from 623 MZ and 377 DZ female Australian twin pairs aged 29-50 years. Univariate twin pair (tetrachoric) correlations of $r_{MZ} = 0.43$ and $r_{DZ} = 0.11$ plus subsequent univariate Cholesky decomposition model-fitting suggested that genetic influences are important in the etiology of CPP. The results of this variance components analysis suggested a heritability estimate of 0.41 (95% CI: 0.25-0.56). However, results of subsequent multivariate model-fitting showed this heritability to be attributable to additional phenotypes significantly correlated with CPP. The results questioned the value of CPP as a useful independent phenotype on which to conduct genetic studies; contributing conditions such as endometriosis and individual variation in perception of pain are likely to provide more useful phenotypes.

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Estimating homozygosity by descent probabilities in large pedigrees. *M. Abney*. Dept Human Genetics, Univ Chicago, Chicago, IL.

Computing the probability of an individual being homozygous by descent (HBD) at a locus is useful when performing homozygosity type linkage analysis in inbred pedigrees. When the pedigree is extremely large, current methods become computationally intractable, particularly when multipoint genotype information is used. Here, I present a method that can perform this computation on pedigrees of extremely large size and arbitrary complexity. I build on my previous method that considered only the genotypes of the individual in question in the hidden Markov model. Now, the computation can be done conditional on the individual's genotypes, as well as all ancestral genotypes, along the chromosome. I demonstrate the method on a genome scan of 500 individuals, all of whom are interrelated via a 13 generation, 1,623 person pedigree. I also assess the accuracy of the method through simulation and compare the results, on smaller pedigrees, to exact methods.

Identification of gene-gene interactions in whole-genome linkage data by the Apriori algorithm. *S. Buervenich¹, J. Ott², F.J. McMahon¹*. 1) Mood and Anxiety Disorders Program (MAP) Genetics, NIMH, Bethesda, MD; 2) Lab Statistical Genetics, Rockefeller Univ, New York, NY.

It is generally believed that multiple interacting genes underlie most complex traits, but there is a lack of powerful analytic tools for detecting such genes. When two or more genes act epistatically to increase disease risk, allele sharing at one of these loci will be correlated with allele sharing at the other. Several techniques have recently been suggested in order to detect correlations of this kind in the human genome. The Apriori algorithm has come into wide use in many fields where fast and efficient data-mining is important, since it can handle large data sets and quickly find positive and negative correlations between any group of data points. Here we apply an implementation of Apriori (Christian Borgelt, 1996-2003) to genetic linkage data. We have 2 goals: 1) to divide the genome into optimal bins based on correlation of linkage evidence at neighboring groups of loci, rather than on arbitrary borders or bin sizes, and to determine the influence of a similarity parameter (that compares the support of each set of bins with each possible subset) on the average size of the resulting bins; 2) to test the power and efficiency of Apriori to detect interacting loci in simulated data from the Genetic Analysis Workshop 11 (GAW11; Greenberg et al.[1999]; supported by grants DK31775 and GM31575). In our preliminary analyses, Apriori was sensitive enough to identify two of the interacting loci in the simulated dataset when using a similarity parameter of 1.4, 5% best lod scores as threshold and 10% minimum support and confidence for any rule. However, sensitivity depended in part on the threshold at which a given family was considered to show linkage at a given locus. Also, specificity has to be addressed by determining the false discovery rate. We conclude that Apriori is a potential tool for binning linkage data and uncovering interacting loci. The binning procedure may also be useful for comparing linkage results across studies, as in meta-analyses. Supported by the NIMH intramural research program and extramural NIMH grant MH44292.

Improvements in a score for combined segregation and linkage analysis of complex traits. *E.W. Daw¹, S.C. Heath², J. Ma¹*. 1) UT MD Anderson CA Ctr, Houston, TX; 2) Centre National de Génotypage, Evry, France.

We have previously developed a score for combined segregation and linkage analysis, the Log of the Posterior placement probability ratio (LOP). We have used this score with the Bayesian Monte Carlo Markov chain (MCMC) techniques implemented in the program Loki (Heath, AJHG, 1997). The LOP is the log of the posterior probability of linkage to a real chromosome divided by the posterior probability of linkage to a randomly generated unlinked pseudo chromosome. The pseudo chromosome is generated with marker allele frequencies and positions based on the marker data available on the real chromosome. Preliminary studies of The LOP have indicated that it can be used to localize genes contributing to variation in complex traits in cases where traditional lod score analysis may fail (Daw et al., Genetic Epidemiology, 24:181-190, 2003). Two limitations of our previous implementation of the LOP were (1) the requirement to write a number of specialized scripts to run a series of programs for each analysis and (2) the dependence of each analysis run on a single realization of the pseudo chromosome. To address the first issue, we have incorporated and automated the generation of the pseudo chromosome within the Loki package, so that pseudo chromosomes are now part of the state space of the sampler. To address the second issue, we have enabled the re-generation of the pseudo chromosome within an analysis run in such a way that updating the pseudo chromosome data is part of a valid MCMC sampler. This has resulted in a procedure to estimate the LOP that is much easier to use and has enabled us to examine what frequency of re-generation of the pseudo chromosome is appropriate. Re-generation at intervals of 100,000, 10,000, and 1000 iterations has been examined. We find that re-generation at intervals of 10,000 iterations appears adequate. (support: CA097855).

Constructing a specialized statistical genetics computing resource: experiences with a Macintosh-based grid-computer. *R. Evans, T.J. Bates, M.M. Barmada.* Human Genetics, Univ Pittsburgh, Pittsburgh, PA.

Statistical genetics requires enormous computing resources to successfully address issues such as empirical p-value estimation, complete multipoint linkage analysis, haplotype frequency estimation, and linkage disequilibrium mapping. Statistical methodologies involving coalescent theory and Markov-Chain Monte-Carlo (MCMC) based simulation methods require long runs and are highly amenable to parallel processing strategies. In order to address these needs, we have previously described our experiences with a distributed grid-based computing system. We have recently extended this computing system dramatically. In implementing this expansion, we choose to work with a new hardware vendor choosing to purchase compute nodes from Apple Computer. Advantages of the move to Apple hardware include: (1) a unique 128-bit wide vector-processing unit; (2) Sophisticated, integrated hardware and software monitoring tools; (3) 64-bit processor architecture, allowing access to 8Gb of RAM per process; (4) Highly optimized C and Fortran compilers; and (5) A ready-to-use cluster and web portal optimized for use in life science informatics settings (iNquiry). As the Macintosh operating system (MacOS) is currently Unix based, moving common statistical genetics programs (merlin, simwalk2, vitesse, pedcheck, aspex, genehunter, allegro, transmit, and mega2, just to name a few) to this platform often requires nothing more than recompiling. Results comparing the performance of these programs on this Macintosh cluster to performance on a Pentium4 Xeon cluster will be presented, to highlight the advantage provided by optimized compilers. Specialized versions of certain programs (merlin, pedcheck, vitesse) that have been modified to take advantage of the Apple vector-processing unit (AltiVec) will be compared as well. As the cluster/web-portal tool that we are using (iNquiry) includes XML templates for the implementation of any command line tool, web-based access to many statistical genetics programs is possible, and will be demonstrated.

Estimation of Haplotype Frequencies with Pedigree Data. *D.K. Folie, D.J. Schaid.* Health Sciences Research, Mayo Clinic, Rochester, MN.

Although there are well established methods to estimate haplotype frequencies from unphased genotype data for genetically unrelated subjects, the current methods for pedigree data are limited. Most pedigree-based methods assume that the markers are in linkage equilibrium, which may not be reasonable for densely spaced markers currently available. Furthermore, many software packages for pedigree analysis output only one pair of (most likely) haplotypes per subject. This can be imprecise, because the ambiguity of haplotypes is not accounted for, and it does not provide information on the joint configuration of possible haplotype pairs for a pedigree. For large pedigrees, where there are an enormous number of configurations of pairs of haplotypes (diplotypes), complete enumeration is impractical. For small to moderate size pedigrees, however, enumeration is feasible, particularly when combined with a trimming of configurations that have small probabilities. We have developed an expectation-maximization (EM) algorithm to estimate haplotype frequencies for data comprised of many pedigrees. The pedigree structures can be fairly general, but without loops. However, memory constraints give practical limits on their sizes. The pedigrees can have multiple founders, and the non-founders can have multiple spouses. The main steps of this algorithm are as follows: 1) use genotype elimination to remove superfluous genotypes at all loci; 2) insert a batch of loci, enumerating all possible diplotypes for all pedigree members; 3) run genotype elimination to retain only non-recombinant haplotypes; 4) use a modified Elston-Stewart algorithm that drags founder diplotypes to the top of the pedigree, resulting in an enumeration of all possible diplotype configurations among founders; 5) use an EM algorithm, which uses pedigree segregation probabilities as weights, to compute haplotype frequencies, along with posterior probabilities of founders diplotype configurations; 6) trim off configurations with small probabilities; 7) repeat steps 2-6 until convergence. This algorithm is illustrated by examples and computational limits will be presented.

Program Nr: 2873 from the 2004 ASHG Annual Meeting

Statistical Analysis of Multiple Related SNPs. *K.E. McDougal, D.R. Moller, M.D. Fallin, Z. Song, G.R. Cutting, ACCESS Research Group.* Johns Hopkins Univ, Baltimore, MD.

A major challenge in association studies is deciding how to evaluate the significance of allele distribution when multiple candidate genes are analyzed. A chi-square analysis can evaluate the significance of the genotype distribution of a single SNP, but with each additional SNP analyzed, the potential for false positives due to chance increases. A Bonferroni correction for multiple testing is often too conservative and inappropriate for correlated data. Our goal was to develop a program using SAS statistical software that would take the relatedness of all SNPs evaluated into consideration when computing a p-value for a specific SNP. This program uses Monte Carlo permutations to create a study-wide threshold that eliminates the need to correct for multiple testing. The utility of this implementation was evaluated via simulation. As expected the effect size threshold was quite low for the uncorrected test and was high for the Bonferroni correction, with the permutation result lying between these. This is shown graphically to highlight the liberal interpretation of a nominal test and the too conservation Bonferroni approach. Using Monte Carlo permutations, we analyzed fifty functional variants of proteins involved in Th2 mediated inflammation in 350 patients with sarcoidosis, a multi-organ inflammatory disease, and 350 age and sex matched controls from the ACCESS (A Case Control Etiologic Study of Sarcoidosis) Study. We found a significant p-value of 0.037 for FCER1B polymorphism E237G. The nominal chi-squared p-value for this polymorphism was 0.0009. FCER1B is the beta subunit for the high affinity receptor for IgE and E237G has been linked to clinical atopy and high levels of IgE. The significance of an association with FCER1B could reflect the Th1/Th2 dysregulation that is characteristic of sarcoidosis. These results suggest that establishing significance using data randomization may be a viable alternative to other methods that require stringent corrections for multiple comparisons and do not take correlated data into account. In addition, our results implicate FCER1B in the pathogenesis of sarcoidosis. Funded in part by the NHLBI.

Multinomial Regression for Haplotype Association Analysis in NEMO. *S.K. Sieberts, A. Kong, D.F. Gudbjartsson.*
deCODE Genetics, Reykjavik, Iceland.

Log-linear regression is a powerful and flexible tool for the analysis of case-control data with more than two response categories. For the purposes of linkage disequilibrium mapping, we model the genotype counts using a log-linear approach for multinomial data. In this flexible framework, we can adjust for covariates and known population stratification and test a wide variety of hypotheses, including the typical null hypothesis in LD mapping of no allele (haplotype) frequency difference between cases and controls. Using familial data, we can also test models of genomic imprinting. For haplotype analysis, when the genotype being modeled is a phased, multilocus genotype, uncertainty often exists due to phase ambiguity and missing data at individual markers. The EM algorithm is used to maximize the likelihood with respect to the regression model parameters in the presence of this uncertainty. In each iteration of the EM algorithm, the fitted genotype probabilities are used to update the population haplotype frequencies, which are used in the E-step to infer the phased genotype probabilities for each individual. The maximization of the full likelihood is more involved than the two step approach, in which the haplotypes are first inferred and then used to fit the regression model. However, because the full likelihood model properly accounts for the uncertainty in the inferred genotypes, computed p-values and parameter uncertainty estimates are valid, which is not true in the two-step approach. These methods are implemented in NEMO (NEsted MOdels), a general tool for association mapping.

An Empirical Bayes Method for Analysis of Quantitative-Trait Loci from Multiple Genome Scans. *K. Zhang¹, H. Wiener², T.M. Beasley¹, V. George¹, C.I. Amos³, D.B. Allison¹.* 1) Section on Statistical Genetics, Department of Biosatistics, University of Alabama at Birmingham, Birmingham, AL; 2) Department of Epidemiology, University of Alabama at Birmingham, Birmingham, AL; 3) Department of Epidemiology, University of Texas, M. D. Anderson Cancer Center, Houston, Texas.

Single genome scans for Quantitative-Trait Loci (QTL) mapping often suffer from the low power and the imprecise estimation of QTL location and effect. As a consequence, the confidence interval for QTL location is often prohibitively large for subsequent fine mapping and positional cloning. Given that a large number of genome scans for the same phenotype have been available, information borrow across multiple linkage studies may allow us to detect the QTL with greater power and to estimate the QTL location and effect more precisely, therefore obtaining narrower confidence intervals. In this paper, we develop an empirical Bayes approach to integrating the same linkage statistic from multiple sib pair studies as well as not to force all studies to have an identical marker map and a common estimated QTL effect. The updated linkage statistic then can be used for the estimation of QTL location and effect. We evaluate the performance of our method using extensive simulations based on actual marker spacing and allele frequencies on chromosome 11 from the NIMH Alzheimers Diseases Genetics Initiative. The results indicate that the empirical Bayes method can account for the between-study heterogeneity and estimate the QTL location and effect more precisely as well as supply narrower confidence intervals than the individual study. We also compare it with the other related methods that have been developed.

A Permutation-Informed Bonferroni Correction for Correlated Tests. *Y. Zhao, B. Dain, C. Brain, B. Salisbury.*
Genaissance Pharmaceuticals, New Haven, CT.

A crucial statistical issue in genetic association analysis is multiple testing adjustment. Various approaches have been adopted to adjust for multiple testing. Here we propose a new method that combines the strength of permutation correction with the simplicity of Bonferroni. If the n markers in a gene were independent, a Bonferroni correction of p -values would simply consist of multiplying the observed p -value by n or dividing the significance cutoff, ALPHA , by n . However, it is almost never true that all the markers in a locus are independent of one another; this is due to linkage disequilibrium and the complex relationships between polymorphisms and haplotypes. Under the null hypothesis of no association between any of the markers in a gene and the phenotype, the expected value of the minimum marker p -value is $1/(1+n)$. We propose the following: Randomly permute the phenotypic data relative to the genetic marker, calculate all p -values and retain the minimum p -value for each permutation, doing enough permutations to accurately estimate the asymptotic average of the minima. The n for which $1/(1+n) =$ the average of the minima is the effective number of independent markers in the locus. This n could then be used in a Bonferroni procedure to accurately correct for the multiple testing. This method was tested on genetic and clinical data from Genaissance Pharmaceuticals STRENGTH study of statin response. The results were compared with those from other commonly used methods. This approach can also be used to correct for the simultaneous testing of multiple genetic models by including those in the permutations to get the effective n for the whole ensemble of tests.

Epidemiological characterization of Congenital Heart Disease in Sao Miguel island, Azores (Portugal). T.

Cymbron^{1, 4}, *R. Anjos*³, *C. Macedo*², *C.P. Duarte*², *L. Mota-Vieira*^{1, 4}. 1) Molecular Genetics & Pathology Unit, and; 2) Pediatric Depart, Hospital Divino Espirito Santo, Ponta Delgada, Azores Islands; 3) Pediatric Cardiology Depart, Hospital Santa Cruz, Carnaxide; 4) Instituto Gulbenkian de Ciencia, Oeiras, Portugal.

Congenital heart disease (CHD) are the clinical manifestation of anomalies in embryonic cardiac development, being the most frequent of all clinically significant birth defects. Here, we present an epidemiological study carried out on the population of Sao Miguel island (Azores, Portugal) during a 10 years-period (January 1992 to December 2001). The children were closely followed up by the only hospital in this island. A total of 189 patients with CHD were diagnosed in Sao Miguel population, indicating an average incidence of 9.20 per 1000 live-birth. During this period the incidence of CHD ranged from 4.77 to 12.75. The most frequent cardiac alterations found were ventricular septal defect (38.1%), atrial septal defect (12.2%), and persistent ductus arteriosus (11.6%). These three lesions amount to 61.9% of all CHD cases. Other lesions present were aortic stenosis (6.9%), atrioventricular septal defect (5.3%), pulmonary stenosis (4.8%), tetralogy of Fallot and pulmonary atresia with VSD (3.7%), coarctation of the aorta and transposition of the great arteries, both with 2.7%. The remaining cardiac malformations showed a frequency below 2%. These data agree in general with other recent studies. The main differences are a higher frequency of persistent ductus arteriosus (11.6%) and atrial septal defect (12.2%), and a lower frequency of transposition of the great arteries. Death occurred in 10.6% (n=20) of the children enrolled in this study. The cardiac lesions with higher mortality were pulmonary atresia with VSD and transposition of the great arteries. Until now, three familial clusters were detected, representing a total of 9 patients, most of which affected with VSD. The data described here represents the first characterization of congenital heart disease in the Azorean population. This work is funded by FCT (POCTI/ESP/49236/2002) and DRCT, Azores, Portugal (lmotavieira@hdes.pt).

Methylenetetrahydrofolate reductase C677T variant and non syndromic cleft lip with/without cleft palate in mestizo Mexican population. *I. Davalos^{1,2}, V. Ledezma-Gomez³, J.P. Mena^{1,2}, V. Ledezma-Rodriguez², E. Ramirez-Lizardo^{2,3}, L. Sandoval¹, N.O. Davalos², M.P. Gallegos¹, M. Salazar-Paramo⁴, L.E. Figuera^{1,2}.* 1) Division de Genetica, CIBO, IMSS; 2) Doctorado en Genetica Humana, Departamento de Fisiologia, CUCS, Universidad de Guadalajara; 3) Instituto Jalisco de Cirugia Reconstructiva Dr. JGS, SS; 4) Unidad de Investigacion Medica en Epidemiologia Clinica, HE, CMNO, IMSS. Guadalajara, Jalisco, Mexico.

MTHFR C677T variant, converting an alanine to a valine residue in the 5,10-MTHFR reductase gene, is responsible of the thermolabile form of the enzyme, reduced availability of 5-methyltetrahydrofolate (circulating form of folate) and mild hyperhomocysteinemia. MTHFR C677T variant has been associated with congenital anomalies such as neural tube defects. The role of the MTHFR-C677T and folic acid in non syndromic cleft lip with/ without cleft palate (CL/P) still controversial. It has been suggested that maternal folic acid supplementation in early pregnancy prevents CL/P. Objective: To determine the frequency of the MTHFR C677T in mestizo Mexican children with non syndromic CL/P. Method: A group of 43 mestizo Mexican children with CL/P (Group CL/P) and a group of 39 normal mestizo Mexican individuals. (Group N) were typed for the MTHFR C677T by the PCR/RFLP (HinfI) method. Genotype frequencies were analyzed (Hardy-Weinberg Expectations, chi square). Results: MTHFR C677T Genotype Frequencies GF %(n) in Group CL/P were CC 32(14), CT 63(27), TT 5(2) and GF in Group N were: CC 33(13), CT 54(21), TT 13(5). MTHFR C677T Allele Frequency AF %(n) in group CL/P were C 64(55), T 36(31) and in group N AF were C 60(47), T 40(31). The AF differences between both groups were NS ($p>0.05$). Conclusions: These results do not support an association between CL/P and MTHFR C677T genotypes in affected children.

Epidemiological study of cleft lip and palate in Cebu City, Philippines. *T. Pawar¹, M. Tolar², M. Tolarova¹.* 1) Craniofacial Genetics - Ortho, UOP Sch of Dentistry, San Francisco, CA; 2) Pediatric Clinical Research Center, PCRC Core Laboratory, Children's Medical Center, UCSF, 505 Parnassus Avenue, San Francisco, CA.

Cleft lip and palate anomalies, including syndromes and multiple congenital anomalies, affect one out of every 550 newborns. Our study population came from the island province of Cebu, Philippines. We looked at 104 individuals affected with orofacial clefts and 76 controls (patients without a congenital anomaly). A spectrum of factors such as sex, age, family history, birth order, age of the mother at the time of birth of the index child, and month of birth were evaluated. The mean age of cleft patients was 7.9 years, a majority of which (72%) had cleft lip and palate. The mean age of controls was 4.26 years. Among unilateral cases, the left side was more often affected (71%). A family history of clefting was positive in 60% of cases. The mean value for birth order was 3.4. A higher (3.9, but not statistically significant) for birth order was found in cases affected with bilateral clefts. The mean maternal age at the time of the child's birth was 27.4 years for cases and 26.1 years for controls. The majority of case mothers (23.91%) fell in the 24-26 years age-group, and the majority of control mothers fell in the 18 to 20 years age-group. A three month weighted average of the month of birth of cases and controls showed two peaks one for the months of May to July and one for the months of September to November. Projected to the time of conception, these peaks showed that a high number of affected individuals were conceived in the months of September to November (end of the rainy season in the region) and in the period from December to February (the dry season). This pilot epidemiological study evaluates several variables that are historically considered as risk factors for congenital anomalies. More extensive analysis of environmental, nutritional, and genetic factors is in progress to determine factors in the etiology of cleft lip and palate in this Southeast Asian region. The fieldwork for this study was supported by Rotaplast International Inc.

Quantitative genetic analysis of blood pressure before, during, and after isometric exercise. *A.C. Choh, S.A. Czerwinski, M. Lee, E.W. Demerath, B. Towne, R.M. Siervogel.* Wright State University School of Medicine, Dayton, OH.

Exaggerated blood pressure (BP) reactivity and diminished BP recovery in response to isometric exercise may be indicative of cardiovascular disease risk. However, characterizing differences between BP at rest, during, and after isometric exercise is difficult because these measures are presumed to be at least partly influenced by common genetic and physiological pathways. This study examines the genetic architecture of BP before, during, and after isometric exercise.

The study population consists of 448 individuals ranging in age from 7 to 72 years, distributed across 4 kindreds from Dayton, OH. Systolic (SBP) and diastolic BP (DBP) were collected while sitting, supine, during 1/3 maximal hand-grip at 1-minute intervals (up to 3 minutes), and during recovery at 2-minute intervals (up to 16 minutes). BP reactivity was calculated as BP during exercise minus BP at rest, while BP recovery was calculated as BP after exercise minus BP during the last minute of exercise. Heritabilities (h^2) were estimated using a maximum likelihood variance components method. Generally, h^2 estimates of BP before, during and after exercise were significant ($p < 0.05$), ranging from 0.18-0.45. However, the h^2 of the change scores (BP reactivity and recovery) were generally not significant.

To reduce the number of variables examined, principal components analysis was performed using mean arterial pressure (MAP, $DBP + 1/3(SBP - DBP)$) on a subset of individuals ($n = 303$). The first principal component (PC1) was interpreted as "general MAP"; PC2 as "reactivity"; and PC3 as "recovery". The h^2 for "general MAP" was 0.49 ($p < 0.05$). For BP "reactivity" and "recovery" h^2 was not significant. Age, sex and hypertensive medication use were significant covariates of "general MAP". Smoking and sex were significant covariates of BP "reactivity", while only smoking was significantly associated with BP "recovery". Unlike other physiological challenges such as the cold pressor test, reactivity to isometric exercise appears not to have a significant genetic basis. Supported by NIH-HL69995, AHA-0325371B.

Genetic and haplotype diversity of the C-reactive protein (CRP) gene in 7,000 Americans. *D.C. Crawford, J.D. Smith, Q. Yi, L. Witrack, M.J. Rieder, D.A. Nickerson.* Dept Genome Sci, Univ Washington, Seattle, WA.

C-reactive protein serves as a general marker for inflammation in humans, and elevated levels are associated with coronary artery disease. To investigate the impact genetic variation has on plasma C-reactive protein levels, *CRP* (6.7kb) was completely re-sequenced in five samples (23 European-Americans, 24 African-Americans, 24 Asians, 10 Mexicans, and 6 Indo Pakistanis) found within the U.S. We identified a total of 35 unique variants in five samples; 19 are unique to one sample. Twenty-three inferred (PHASEv2.0) haplotypes were identified, of which 2 were found in all five samples representing 47% of the chromosomes surveyed. *CRP* haplotype heterozygosity was highest in African-Americans (0.86), followed by Indo Pakistanis (0.75), European-Americans (0.75), Mexicans (0.74), and Asians (0.64). We then applied a subset of these variations (tagSNPs) to DNAs collected during the third National Health and Nutrition Examination Survey (NHANES III), a representative sample of the U.S. population conducted by the Centers for Disease Control and Prevention between 1988 and 1994. NHANES III has an extensive laboratory component that includes serum levels of C-reactive protein. DNAs from NHANES III (60% European-Americans and 30% African-Americans by self-reported race; 30% Mexican by self-reported ethnicity) were genotyped by TaqMan for 5 previously validated tagSNPs (LDSelect; $r^2 > 0.64$). On average, 94% of the 7,296 NHANES III samples were genotyped successfully. Haplotypes were inferred using all 5 tagSNPs genotyped in NHANES III and the five re-sequenced samples. All six tagSNP haplotypes observed in the re-sequenced samples were observed in NHANES III. Also, 11 rare (<5% frequency) tagSNP haplotypes were identified in NHANES III. Inclusion of the rare tagSNP haplotypes does not substantially increase tagSNP haplotype heterozygosity (0.79) compared with the re-sequenced sample (0.77). These results demonstrate the utility and necessity of exhaustive variation discovery in candidate genes prior to tagSNP selection for large epidemiological studies and serve as a primer for genotype-phenotype correlations for C-reactive protein in NHANES III.

Replicate evidence for intragenic and intergenic interactions between SNPs within the *APOA1/C3/A4/A5* gene cluster. *S.C. Hamon*¹, *S.L.R. Kardia*², *K.L. Klos*³, *K. Liu*⁴, *A.G. Clark*⁵, *E. Boerwinkle*³, *C.F. Sing*¹. 1) Department of Human Genetics, University of Michigan, Ann Arbor, MI, USA; 2) Department of Epidemiology, University of Michigan, Ann Arbor, MI, USA; 3) Human Genetics Center, University of Texas Health Science Center, Houston, TX, USA; 4) Department of Preventive Medicine, Northwestern University, Chicago, IL, USA; 5) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY, USA.

Apolipoprotein metabolic interactions are motivating the examination of SNP-SNP interactions. We considered 80 SNPs within the *apolipoprotein A1/C3/A4/A5* gene cluster using an over-parameterized general linear model to identify SNP pairs whose effects interact to influence plasma total cholesterol (TC). We analyzed population-based samples of unrelated 18 to 30 year old African-Americans (n=1858) and European-Americans (n=1973) ascertained without regard to health in four field centers (Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA) by the Coronary Artery Risk Development in Young Adults (CARDIA) study at the baseline time point (1985-1986). The effects of eight pairs of SNPs, involving nine SNPs from across the gene cluster, combine nonadditively to influence TC levels in females (p 0.05). Differences in mean TC levels of two-SNP genotypes replicated across ethnic groups and across field centers within ethnic groups. In contrast, only one of the 80 SNPs had a statistically significant influence on TC levels (p 0.05) in females within both ethnic groups. Our analyses suggest that SNP effects within the *APOA1/C3/A4/A5* gene cluster interact to influence TC levels in young adults and that this influence is contingent on the context defined by gender. Ignoring SNP-SNP interactions when modeling genotype-phenotype relationships may result in failing to detect the contribution of genetic variation to phenotypic differences in traits such as TC. This research was supported by NIH grants GM065509, HL072904, HL072905, HL072810, HC048047, HC048048, HC048049, HC048050 and HC095095.

Identification of Novel Polymorphisms in the Human ACAT2 Gene and Association Study with Plasma Lipids and Coronary Artery Disease. X. He¹, Y. Lu², N. Saha¹, H. Yang², C. Heng¹. 1) Paediatrics, National University Singapore, Singapore; 2) Biochemistry, National University Singapore, Singapore.

Acyl-CoA cholesterol Acyltransferase-2 (ACAT2) plays a critical role in the development of diet-induced hypercholesterolemia and atherosclerosis. In order to investigate the effects of ACAT2 gene on coronary artery disease (CAD) risks and plasma lipids, this gene was screened for sequence variations using cord blood from 336 neonates by the combination of PCR, denaturing-high performance liquid chromatography (DHPLC) and DNA sequencing. A total of 11 polymorphisms were detected, 9 of which were novel. There were 3 non-synonymous cSNPs: two common SNPs and one rare SNPs. These two common cSNPs (E14G in exon 1 and T254I in exon 7) and a 48-bp insertion/deletion were genotyped using PCR and/or RFLP analysis in 633 CAD patients and 813 healthy controls in Singapore population, namely, Chinese, Malays and Asian Indians. In order to observe the influence of these two polymorphisms on lipid profiles, the healthy subjects were further stratified into normolipidemic and dyslipidemic groups according to the NECP ATP III criteria. We found a significant decrease in the minor I allele frequency of T254I in the Chinese CAD patients compared with that in the controls (0.20 vs. 0.26, $P = 0.01$). It seems that I allele is protective against CAD in Chinese. This was not consistently observed in the Indians and Malays. The heterozygous carriers of T254I have statistically significantly decreased anti-atherogenic ApoA1 ($P=0.027$ and $P=0.007$, respectively) and/or increased ApoB ($P=0.007$) and lipoprotein (a) [Lp(a)] ($P=0.011$) than non-carriers in normolipidemic female Indians and Malays ($P=0.007$). Likewise, the heterozygous carriers of E14G was associated with higher total cholesterol ($P=0.042$) in the same Indian groups. T254I was in nearly complete linkage disequilibrium (LD) with Indel (> 0.9) and very strong LD with E14G (> 0.5) in the healthy three ethnic groups. Associations similar to T254Is were observed between Indel and lipids profile. Our results suggested that these three polymorphisms are associated with plasma lipids and CAD risks in an ethnic- and gender-specific manner.

The Effect of Stratification on Familial Dimensions in ASD. *P. Szatmari¹, S. Bryson², L. Zwaigenbaum³, W. Roberts⁴, S. Georgiades¹, E. Duku¹.* 1) Department of Psychiatry & Behavioural Neurosciences, McMaster University; 2) Departments of Pediatrics and Psychology, IWK Health Centre-Dalhousie University; 3) Department of Pediatrics, McMaster University; 4) Department of Pediatrics, The Hospital for Sick Children, University of Toronto.

Background: One explanation for the discouraging results of recent genetic studies in the Autism Spectrum Disorders is that the traditional DSM-IV phenotype may not be the most informative for genetic purposes. **Objectives:** to identify familial dimensions to be used as quantitative traits in linkage analysis by: a) developing empirically derived dimensions to represent the ASD phenotype, b) estimating the familial aggregation of these dimensions and c) using sample stratification to examine the heterogeneity of familiarity estimates. **Method:** Our sample consisted of 339 individuals with ASD. Four dimensions based on items from the ADI-R were derived using Principal Components Analysis (PCA). The VABS Adaptive Behaviour Composite was added as a fifth dimension to measure level of functioning (LOF). Sample stratification was applied using verbal/non-verbal status as the covariate. Intraclass Correlations (ICCs) were calculated for each dimension and across all sub-samples. **Results:** The four dimensions derived from PCA include: social communication, language, insistence on sameness (IS), repetitive sensory and motor behaviour (RSMB). Familial aggregation exists among sib pairs for all dimensions except RSMB. When the sample is stratified according to verbal or non-verbal status, the familial aggregation for the IS dimension is familial only in the verbal sub-sample. The RSMB dimension becomes familial in the nonverbal sub-sample. The familiarity of the level of functioning dimension appears to be stable across sub-samples. **Conclusions:** The five dimensions are potentially informative for genetic studies, and may be a preferable approach to the current phenotype definition represented in the DSM-IV. In addition, the use of empirical methods such as PCA combined with sample stratification methods are useful techniques for the development of informative quantitative phenotypes that can be used in genetic studies.

ASSOCIATION STUDY BETWEEN (AC)_n MICROSATELLITE POLYMORPHISM OF ALDOSE REDUCTASE GENE AND DIABETIC NEPHROPATHY IN TUNISIAN TYPE 2 DIABETIC PATIENTS. A.

Abid¹, I. Arfa², C. Amrouche-Rached¹, H. Elloumi-Zghal², B. Kaabi², S. Chakroun², S. Blousa-Chabchoub¹, K. Dellagi², S. Abdelhak². 1) Diabetology A, National Institut of Nutrition ,Tunis, Tunisia; 2) Immunology,Institut Pasteur, Tunis, Tunisia.

Aldose reductase (ALR2), the enzyme of the polyol pathway may play an important role in the pathogenesis of diabetic microvascular complications namely diabetic nephropathy. In an attempt to examine the contribution of ALR2 microsatellite polymorphism in susceptibility to diabetic nephropathy in Tunisian, a case control study was conducted among 50 type 2 diabetic patients with nephropathy and 30 diabetic patients without nephropathy in spite of a diabetes duration overt 10 years. Association studies between the (AC)_n microsatellite polymorphism localised at 2.1 Kb upstream the ALR2 promoter and diabetic nephropathy have yielded conflicting results. We identified 9 alleles in the studied diabetic population. The putative protective allele Z+2 showed an association with the diabetic group without nephropathy when compared with the diabetic nephropathy group ($\chi^2 = 5.96$, $p = 0.01$, $OR = 0.29$). These findings support the hypothesis that the polymorphism at the 5' end of the ALR2 gene contributes to the susceptibility to diabetic nephropathy in the Tunisian type 2 diabetic patients.

LACK OF ASSOCIATION BETWEEN GENETIC POLYMORPHISM IN THE RENIN ANGIOTENSIN SYSTEM AND DIABETIC NEPHROPATHY IN TUNISIAN TYPE 2 DIABETIC PATIENTS. *I. Arfa¹, A. Abid², C. Amrouche-Rached², H. Elloumi-Zghal¹, B. Kaabi¹, S. Chakroun¹, S. Blousa-Chabchoub², K. Dellagi¹, S. Abdelhak¹.*
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Diabetic nephropathy is a renal complication due to uncontrolled diabetes and genetic predisposition. It can be caused by an increase of glomerular pressure due to diabetes, the glomerular pressure is controlled by the renin angiotensin system. The genes encoding renin angiotensin system components are potent candidate genes namely ACE encoding the angiotensin converting enzyme and AGT encoding angiotensinogen. Several studies described that an insertion/deletion (I/D) polymorphism in intron 16 of ACE gene is associated with plasma and cellular ACE levels, it has also been suggested that AGT gene polymorphism is associated with plasma AGT levels. A case/control study was conducted among 59 Tunisian type 2 diabetic patients with nephropathy and 36 diabetic patients without nephropathy for ACE gene polymorphism and among 43 diabetic patients with nephropathy versus 33 diabetics without renal complication for AGT gene polymorphism. Diabetic controls without nephropathy have a diabetes duration over 10 years. The diagnosis of nephropathy was confirmed at least on two consecutive occasions on urinary albumin excretion. The other causes of albumin excretion were excluded. The I/D polymorphism of ACE gene was analysed with nested PCR in order to avoid mistyping heterozygotes individuals and the M235T polymorphism of AGT gene was analysed using PCR and allele specific restriction. There were no significant differences in allele or genotype distribution between diabetic patients with or without diabetic nephropathy for ACE and AGT genes polymorphisms. From these findings, it was concluded that these two polymorphisms are not related to the susceptibility to diabetic nephropathy in the Tunisian type 2 diabetic patients.

Incorporating compartmental models into statistical genetic models of type 2 diabetes. *Z. Guan, D.V. Conti, R.M. Watanabe.* Dept Preventive Med, Keck School of Medicine at USC, Los Angeles, CA.

In traditional statistical genetic modeling, the penetrance function linking genetic variation to phenotype typically takes a probabilistic form formulated around population-based information, such as disease risk. Detailed biologic knowledge (physiologic, cellular, or molecular) is used to derive the penetrance function, but is rarely incorporated into the function per se. We attempt to inject known biology underlying genotype-phenotype relationship in type 2 diabetes (T2DM) by incorporating a compartmental model (CM) of glucoregulation into statistical genetic models. We developed a CM capable of simulating oral glucose tolerance tests (OGTT), the most commonly used clinical protocol to assess glucose metabolism. Because our CM is physiologically based, each model parameter can be assigned to specific biologic function. As such, genes can be assigned to model parameters to simulate specific genetic defects in the glucoregulatory system. We performed simulations in which the peroxisome proliferator-activated receptor-2 (PPARG2) gene, a T2DM susceptibility gene known to alter insulin resistance, is imposed on our CM via parameter p3, a determinant of insulin signaling transduction. Mean and variance estimates and biologic limits for p3 were derived from human data. The common P12A polymorphism in PPARG2 was simulated with P corresponding to lower glucose tolerance with an allele frequency of 85%. Our CM was used to simulate 1000 OGTTs assuming an additive genetic model. AA genotype OGTT profiles fell tightly into the normal range. In contrast, the PA and PP genotype groups show considerable variability in glucose profiles, with 4% of the PA genotype having 2-hour glucose >140 mg/dl and 16% of the PP genotype group had elevated 2-hour glucose. The results are consistent with expectation based on the imposed PPARG2 risk profile and demonstrate how a physiologically based CM can replace the standard penetrance function to simulate phenotype data. Additional simulations examining other single gene and multiple gene effects are in progress.

Methylenetetrahydrofolate reductase gene C677T and A1298C polymorphisms and Neuropathy. *I. Kara¹, A. Sazci², E. Ergul², T. Yilmaz³, G. Kaya¹.* 1) Dept Neurosciences, Istanbul Univ, 34280, Istanbul, Turkey; 2) Department of Medical Biology and Genetics, Faculty of Medicine, University of Kocaeli, Derince, 41900, Kocaeli, Turkey; 3) Department of Internal Medicine, Clinics of Diabetes, Faculty of Medicine, University of Istanbul, 34280, Istanbul, Turkey.

Although the causes of diabetic neuropathy are multifactorial, limited data are available on what determine it. Polyneuropathy presents frequent and severe complication of Type 2 diabetes mellitus. The underlying etiopathophysiological mechanisms in diabetic polyneuropathy are multifactorial, and not well-defined. Homocysteine is implicated in the mechanisms underlying alterations in vascular endothelial cells, thus leading to atherosclerosis and venous thrombosis and elevated homocysteine levels may also cause production of oxidation products, thus damaging endothelium by alteration of connective tissue. The MTHFR C677T polymorphism is known to increase homocysteine levels in plasma. Therefore, we examined the MTHFR C677T and A1298C polymorphisms in association with neuropathy in patients with Type 2 diabetes mellitus. In a case-control study, we analyzed the allele and genotype frequencies of 178 diabetic patients with neuropathy and 253 healthy controls using a PCR-RFLP method. The allele frequency of MTHFR 677T was 33.43% in patients and 32.21% in controls. The frequencies of the MTHFR C677C, C677T, T677T genotypes were 44.9%, 43.3%, and 11.8% in patients, and 45.5%, 44.7%, and 9.9% in controls, respectively. The allele and genotype frequencies were not significantly distributed ($\chi^2=0.412$; $df=2$; $P=0.814$). The frequency of the MTHFR 1298C allele was 34.27% in patients, and 29.64% in controls. The allele frequency was not significantly distributed ($\chi^2=2.180$; $df=2$; $P=0.336$). The frequencies of the MTHFR A1298A, A1298C, and C1298C were 42.1%, 47.1%, and 10.7% in patients and 49.0%, 42.7%, and 8.3% in controls. In conclusion, The MTHFR C677T and A1298C polymorphisms are not associated with neuropathy in our study.

Variation in the HNF4A gene may interact with physical activity to increase risk of type 2 diabetes in Hispanic Americans from Colorado. *J.M. Norris¹, T.E. Fingerlin¹, C.D. Engelman¹, M.J. Durfee¹, L.K. Moss¹, M.M. Barmada², R.E. Ferrell².* 1) University of Colorado Health Sciences Center, Denver; 2) University of Pittsburgh, PA.

Variation in the HNF4A gene has recently been associated with type 2 diabetes mellitus (T2DM) in Finnish (FUSION) and Ashkenazi Jewish populations. We typed one of the SNPs shown to increase risk of T2DM in the original reports, rs1884614, in 277 Hispanic American and 252 Caucasian American families from the Denver and San Luis Valley areas in Colorado. We typed a total of 1028 (597 affected, 431 unaffected) Hispanic American individuals and 709 Caucasian American individuals (422 affected, 287 unaffected). Allele frequencies were very similar to those reported for the Finnish and Ashkenazi samples in our Caucasian sample. In contrast, the minor T allele was more common in our Hispanic sample than in the Finnish and Ashkenazi cases (.47 vs. .20 and .27, respectively). We tested for linkage and association between rs1884614 and T2DM using a generalization of the TDT as implemented in the FBAT software. We did not detect association in either population ($p=.10$, $.15$ for Hispanics, Caucasians, respectively). The original FUSION report showed association between the risk allele of rs2144908, a marker in nearly complete LD with rs1884614 in Finns, and insulin secretion measures. Since physical activity is associated with insulin secretion, we subsequently investigated the interaction between total physical activity and HNF4A genotype. We grouped cases based on sex-specific tertiles of total METs/wk prior to T2DM diagnosis and tested for association separately in the top and bottom tertiles. The T allele of rs1884614 was associated with T2DM in the low physical activity tertile ($p=.033$) but not in the high tertile ($p=.83$) in the Hispanic population. We did not see any such association in Caucasians, but the number of informative families in each tertile was small for that group. We are currently investigating other analytic approaches to verify the interaction between physical activity and HNF4A in conferring risk to T2DM in the Hispanic population.

Investigation of the familiarity of Diabetes Mellitus using Utah population-based mortality data. *B. Tausch*¹, *C.Q. Edwards*^{2,4}, *L.A. Cannon Albright*^{3,4}. 1) Dept of Medical Informatics, Univ of Heidelberg, Heidelberg, Germany; 2) Dept of Medicine, Univ of Utah, Salt Lake City, UT; 3) Dept of Medical Informatics, Univ of Utah, Salt Lake City, UT; 4) LDS Hospital, Salt Lake City, UT.

We used the the Utah Population Database (UPDB) to study the familial/genetic nature of diabetes mellitus (DM). The UDPB is a unique data resource that contains genealogical information of the Utah population. Therewith it is possible to examine all known genetic relationships between all DM deaths. Computerized data from Utah death certificates since 1904 have been record-linked to the genealogy data, which allows assessment of the familial contribution to disease.

We analyzed data from 14,000 individuals whose genealogical data is contained in the UPDB and whose death certificates indicated death due to DM Type 1 (T1DM) or Type 2 (T2DM). We used our previously published methods to examine the familial/genetic nature of DM, including birthyear- and sex-specific rates to estimate relative risks (RR) for DM in first- and second-degree relatives, by DM type. We also used the Genealogical Index of Familiarity (GIF), a published method that was developed by our group, to test for excess of relatedness among DM deaths. BMI data from drivers' licenses, which were also linked to the UPDB, allowed us to analyse the case group by obesity status. Our results strongly support significantly increased first- and second-degree RR for both T1DM and T2DM, analyzed independently. We observed significantly increased first- and second-degree RR for T2DM among the relatives of T1DM probands. This suggests a relationship between the phenotypes which may represent a common predisposition. We observed significantly increased RR for T2DM among the first-degree relatives of the normal, overweight, and obese weight subgroups, as well as among the second-degree relatives of the normal weight group. These results support the hypothesis that relatives of DM patients are at elevated risk for DM, independent of obesity status. We identified sets of high-risk pedigrees showing significant excess of DM, which could prove useful for predisposition gene searches.

Variation in PPARG associated with insulin sensitivity response to Troglitazone does not account for analogous response to Pioglitazone. *J.K. Wolford¹, K.A. Yeatts¹, S.K. Dhanjal², A.H. Xiang², T.A. Buchanan², R.M. Watanabe².*
1) Translational Genomics Research Institute, Phoenix, AZ; 2) Keck School of Medicine, University of Southern California, Los Angeles, CA.

We previously reported association between variation in the peroxisome proliferator-activated receptor gamma 2 (PPARG2) gene and response to troglitazone (TRO), a PPARG2 agonist and insulin-sensitizing agent used to treat type 2 diabetes. The association between PPARG2 and response to TRO was assessed in Hispanic women with previous gestational diabetes mellitus participating in the Troglitazone in the Prevention of Diabetes (TRIPOD) study, a placebo-controlled TRO trial. The recall of TRO for clinical use resulted in a new 3-year, single-arm, open-label Pioglitazone (PIO) trial, the Pioglitazone in the Prevention of Diabetes (PIPOD) study. Women who previously participated in the TRIPOD study were invited to participate in the PIPOD study. Oral (OGTT) and intravenous glucose tolerance tests (IVGTT) were performed at baseline, 1-year, and 3-years post-treatment. Non-response to PIO was assessed as a non-significant change from baseline insulin sensitivity (SI) after one year of treatment. Data are currently available for 78 PIO-treated women: 46 who took placebo and 32 who took TRO in TRIPOD. Non-response to PIO was 40%, slightly higher than that observed in TRIPOD. Only 19 of the 32 women (60%) who received active drugs in both studies showed concordance for response: 16 concordant for response, 3 concordant for non-response. We sequenced ~40 Kb of PPARG identifying 125 SNPs and 6 insertion/deletions. The six PPARG2 variants that were associated or showed trend for association ($p < 0.08$) with response to TRO were genotyped in PIPOD subjects. None of the six variants showed association with response to PIO ($p > 0.10$), nor did they show association with changes in response-related phenotypes. We conclude that variation in PPARG2 that may account for response to TRO does not account for response to PIO. Our results suggest other variants within PPARG2 or other genes in the thiazolidinedione-stimulated pathway may be responsible for response to PIO.

Large-scale epidemiological study of polymorphisms predisposing for common diseases in the Swedish

population. *U. Hannelius*^{1,2}, *C.M. Lindgren*^{1,2}, *E. Melén*^{2,3}, *A. Malmberg*⁴, *U. von Döbeln*⁴, *J. Kere*^{1,2}. 1) Department of Biosciences, Karolinska Institutet, Huddinge, Sweden; 2) Clinical Research Centre, Karolinska University Hospital, Sweden; 3) Institute of Environmental Medicine, Karolinska Institutet, Sweden; 4) Center for newborn metabolic diseases, Karolinska University Hospital, Sweden.

Neonatal screening has given rise to large libraries of biological samples in the form of dried blood spots. Our aim is to validate a method that makes these sample resources available for large genetic studies without violating the individual integrity or wasting the precious material. DNA from untraceable Guthrie spots was extracted using a saponin and chelex-100 based method. DNA yield was fluorometrically determined using OliGreen and the samples were preamplified using I-PEP-L. Genotyping was performed using the Sequenom MassArray platform. Ten samples each of fresh, 10 year-old and 25 year-old samples were extracted and preamplified. The fresh and the 10 year-old samples gave DNA yields of 105ng (SD36) and 96ng (SD37) respectively, whereas the 25 year-old samples gave yields of 57ng (SD19). After preamplification the samples were subjected to PCR for amplicons of 100, 396, 499, 599 and 762 bp in length. The 25 year-old samples were 100% successful for the 100bp amplicons and 80% successful for the 396bp amplicons. The 10 year-old samples were 100% successful for the 100bp and 396bp amplicons, 90% successful for the 499bp and 80% successful for the 599bp amplicons. The fresh samples were 100% successful for all amplicon lengths. 94 fresh samples including an unknown number of carriers of two different PKU mutations (R158Q, R252W) were genotyped in order to validate method specificity. All carriers (6 R158Q carriers, 4 R252W carriers) were found with no false positives. To determine the population frequency of several susceptibility genes we extracted and preamplified 2047 Guthrie spots representing the Swedish population. We describe a method that makes use of a preamplification step to use PKU registries for large genetic studies in an economical and ethical way. We also show that the approach is specific and applicable on old samples.

Methods for distinguishing mediating versus moderating forms of gene-environment interactions. B.M.

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Gene-environment (GxE) interaction effect on specific phenotypes is defined by heterogeneity of environmental (E) effects across different genotypes, or by genotypes (G) having different phenotypic effects under different environmental conditions. Theoretically G x E effects may exist in spite of the absence of main effects of G and/or E. Thus, mechanistic distinctions of different forms of G x E interaction effects can be made by utilizing the concepts of mediator and moderator, used extensively in psychological and sociological studies. Structural equation models, or path diagrams describe the characteristic features of these two forms of interactions, which in turn allow their discrimination. Analysis of variance (ANOVA), or multiple/partial regression analysis (MRA/PRA) is the method of choice for data analysis, employed for different scenarios of continuous or discrete measurements of phenotypic, genotypic, and environmental variables. Necessary and sufficient conditions for moderator/mediator distinctions are derived in terms of the parameters of the ANOVA and MRA/PRA models. Parameterization of moderator/mediator forms of G x E effects also allows formal power computations for designing studies of gene-environmental interactions. With data on the effect of lead exposure on postural balance (an indicator of neuromotor function) in children of the Cincinnati Lead Program Project (CCLP), it is shown that impaired neuromotor balance under lead exposure is moderated by genotypes at a number of genetic loci implicated in susceptibility to neurological diseases. (Genotype data collected for this research was supported by a pilot project of the NIEHS grant ES-06096).

Impact of regulatory polymorphisms in genes involved in cell cycle control on the susceptibility to childhood leukemia. *J. Healy, A. Moghrabi, D. Sinnett.* Sainte-Justine Hospital Research Center, University of Montreal, Quebec, Canada.

Acute lymphoblastic leukemia (ALL) is the most frequent neoplasia in children, accountable for approximately 25% of all pediatric cancer cases. Sporadic cancers, such as ALL, are complex genetic disorders in which the effect of a series of low penetrance genes is modulated by external factors, hence modifying ones individual risk of cancer. This model implies an important interplay between genetic and environmental factors that influences inter-individual variability in the susceptibility to cancer. We wish to study the genetic determinants involved in childhood leukemogenesis, our hypothesis being that a child's individual susceptibility to ALL is associated to polymorphisms in genes involved in cell cycle control, which is crucial for maintenance of cellular homeostasis and genomic integrity. The main objective of this genetic epidemiology study is to evaluate the impact of polymorphisms in the 5' regulatory region of selected candidate genes on the susceptibility to ALL. In order to determine an association between genotype and clinical and/or molecular phenotype, 258 ALL patients and 277 control individuals, as well as 147 parental trios (father-mother-patient) were genotyped using a PCR and allele-specific oligonucleotide hybridization based approach. Preliminary results of our case-control and family based association studies have revealed that the A-593T polymorphism in CDKN2B, an important cell cycle inhibitor, is associated with ALL. The T-593 allele was shown to be significantly under-represented in ALL patients, suggesting a protective effect among carriers (OR=0,768; p=0,048). Similar studies on other candidate genes are ongoing. Our study focuses on individuals of French Canadian origin however the general population, including adult cancer patients, could benefit from the outcome of this study. Ultimately, our study will provide the ground work to understanding the role of genetic susceptibility factors, particularly those pertaining to the cell cycle, in the etiology of childhood ALL. This study is supported by the Francois-Karl Viau Chair in Pediatric Oncogenomics.

Segregation analysis of cancer in families of young onset African American breast cancer patients. *J.F. Korczak, M.S. Simon, C.L. Yee, A.G. Schwartz.* Karmanos Cancer Inst. and Wayne State Univ., Detroit, MI.

Breast cancer etiology has been understudied in young African American women, where the disease incidence and mortality exceed that of Caucasians. We recently found significant familial risk of breast and cervical cancer in 3,037 female first-degree relatives of 496 African American breast cancer patients diagnosed before 45 years of age who participated in the NICHD-sponsored Women's Contraceptive and Reproductive Experiences (CARE) Study.

To determine whether the transmission of a single major gene could account for the increased familial risk of these cancers, we considered anyone with (i) breast cancer (BR) or (ii) breast or cervical cancer (BRCV) as affected and performed complex segregation analyses of BR and BRCV using Class A logistic regressive Models 1 (type-dependent age of onset) and 2 (type-dependent susceptibility), as implemented in the computer program S.A.G.E. REGTL. For each model, hypotheses of a major genetic or environmental effect, as well as no major type effect, were tested against a general model with arbitrary transmission probabilities.

According to the Akaike Information Criterion, Model 1 fit the data as well as or better than Model 2 for all transmission hypotheses, both for BR and BRCV. The genetic Model 1 hypotheses tended to have larger P -values than the corresponding non-genetic hypotheses, especially for BRCV where P was greater than 0.01 for the genetic hypotheses but less than 0.0003 for the non-genetic hypotheses. This suggests that BRCV may have greater power to discriminate among alternative transmission hypotheses than BR. All Model 1 transmission hypotheses of BR and BRCV were rejected at the $\alpha = 0.05$ level when tested against the general model, leading us to conclude that the mode of transmission may be more complicated than a simple single gene model. We are thus evaluating the utility of several additional parameters in the above models to allow for departure from a logistic distribution of the age at exam and/or age at onset, via a Box-Cox transformation, and for residual familial correlations, via a regressive maternal effect and/or sibship covariate.

Evaluating interaction between cigarette smoking and genetic susceptibility in carcinogenesis of lung cancer using a new carcinogenesis model and case-control data. *L. Deng¹, O. Gorlova², M. Kimmel¹*. 1) Department of Statistics, Rice University, 6100 Main St. MS-138, Houston, TX 77005; 2) Department of Epidemiology, University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030.

The majority of lung cancer cases are attributed to tobacco. However, only a fraction of smokers will develop lung cancer in their lifetime. This suggests that the probability to develop lung cancer is affected also by genetic susceptibility including the ability to activate the carcinogen, detoxify the carcinogen and repair damaged DNA. We present a new carcinogenesis model and apply it to the simulated case-control data in order to make inferences on the key parameters. Our carcinogenesis model inherits some features of the two-stage clonal expansion and multi-stage models. However, it also incorporates a mechanism of cancer cell evolution, which allows different carcinogenesis pathways for different patients. This is accomplished by introducing a continuous malignancy parameter, which is incremented in steps of random durations. The pace of transformation accelerates with increasing malignancy. The resulting incidence curves are initially very flat and then rapidly increase, this biphasic character being more pronounced than in Moolgavkar-style models. We will compare the performance of our new model with Moolgavkar's carcinogenesis model using Texas Lung Cancer Registry data. We also expect that the data containing information on individual genetic make-up from an ongoing case-control study at M.D. Anderson Cancer Center will be made available to us through collaboration. This data will allow evaluating the interaction between smoking and genetic factors in the carcinogenesis of lung cancer. The research is supported by NCI grants CA97431 and by a training fellowship from the W.M. Keck Foundation to the Gulf Coast Consortia through the Keck Center for Computational and Structural Biology.

The relationship between parity and longevity in the Old Order Amish, a population characterized by large family sizes. *P. McArdle, T. Pollin, A. Shuldiner, B. Mitchell.* School of Medicine, University of Maryland, Baltimore, MD.

From theoretical perspectives, there is reason to speculate that having children might lead to decreased life span. To evaluate this issue, the authors examined the relationship between parity and longevity using genealogical data from an Old Order Amish community in Lancaster, PA, a traditional founder population who do not believe in divorce or birth control. We restricted our analysis to the set of 2338 individuals born between 1749 and 1912 and surviving until at least age 50 years. Individuals who had at least one child lived longer than those who did not though there is greater evidence among men (75.2 vs 73.3 years, $p=0.05$) than among women (76.4 vs 76.1 years, $p=0.7$). Among the 2015 individuals who had children, the average number of offspring was 7.3, with a range of 1 to 20 children. In fathers, life span increased significantly and in linear fashion with each additional child ($p=0.01$). In mothers, life span increased with increasing parity until about 11-14 children ($p=0.004$), but tended to decrease thereafter in those having more than 14 children. Among Amish women, age at last birth was independently associated with longevity, even after accounting for total number of children, with women who gave birth later in life living longer ($p=0.0001$). Late childbirth in women thus appears to be a marker for increased longevity, suggesting that genetic or environmental factors beneficial to reproductive health are also positively associated with longevity. Understanding these factors may provide insights into mechanisms underlying successful aging.

A Novel Quantitative Trait (QT) Posterior Probability of Linkage (PPL) with Specific Adaptations for QT Analysis of Autism. *C.W. Bartlett, V.J. Vieland.* Center Stat Genet Res, University of Iowa.

We have developed a new method for QT linkage analysis based on the PPL, which directly measures the probability that a disease-gene is linked to a genetic marker, using the single-locus QT likelihood implemented in LIPED with the trait parameters (allele freq, genotypic means, variances) integrated out. This framework has several advantages over pair-wise IBD sharing-based methods: it is based on the full likelihood, uses full phenotypic information, does not assume normality at the population level or require population/sample parameter estimates; and like other forms of the PPL, it is specifically tailored to accumulate linkage evidence either for or against linkage across multiple sets of heterogeneous data. Simulation evaluation of the method confirms its utility compared to other approaches and also confirms that the single-locus likelihood is robust for two-locus traits. E.g., in data of 100 nuclear families ascertained for 2 children with phenotype >1 SD above the population mean (locus effect size 0.3), we obtain mean PPLs as follows: additive-additive model, 50%, 51%; respectively for the two loci; additive-dominant, 58%, 36%; additive-recessive, 21%, 99%. These are similar to the values we obtain for comparable single-locus traits (additive 56%, dominant 45%; and recessive 100%).

We have also modified the QT PPL by adapting the threshold model to handle a mix of quantitative and dichotomous data within the same pedigree, as occurs when QT measurea can only be obtained, or appropriately normed, for clinically unaffected individuals. Repeating our simulations by recoding all QTs >1 SD above the mean as affected and using the modified likelihood, the mean PPL is reduced by only 12%. By contrast, using the same threshold but a dichotomous trait PPL reduced the mean by 30%, strongly supporting the use of this new approach when QT measures are available for a subset of individuals only. This adaptation of the PPL has been tailored to genetic studies of diseases such as autism, where quantitative endophenotypes may be measurable only in relatives, and not in severely impaired autistic individuals.

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Association explaining non-parametric linkage for binary traits in general pedigrees. *L. Li, S.R. Browning.* Genetic Data Sciences, GlaxoSmithKline, RTP, NC.

A successful linkage analysis may yield a gene-mapping signal covering a large region of perhaps thirty centiMorgans in length and containing hundreds of genes. A follow-up association analysis can provide much finer resolution but is subject to greater multiple testing problems. When several weak association signals are found within a linkage region, a question of great interest is whether there is any way to distinguish the false from true positives. We present a test for determining whether an association result can help to explain a non-parametric linkage result for a binary trait in general pedigrees. The correlation between family allele/genotype frequency and family LOD score is used as a measure of whether the association between a given marker and the disease status can help to explain a significant linkage result seen in the collection of families in the region around the marker. Results from simulation studies examining type I error and power indicate the test is valid and useful for distinguishing between true and false positive association results under a linkage peak. We apply the method to analysis of real data.

Association of polymorphisms in pituitary growth hormone and IGF-1 with fetal growth restriction. *R. Adkins*¹, *T.K. Boyd*². 1) Dept Pediatrics, Univ Tennessee, Memphis, TN; 2) Dept Pathology, Children's Hospital Boston, Boston, MA.

We surveyed single nucleotide polymorphism (SNP) and microsatellite variation in the genes for pituitary growth hormone (GH) and insulin-like growth factor 1 (IGF-1) in a case-control analysis of 128 normal birth weight and 72 fetal growth restricted (FGR) full-term newborns selected using stringent inclusion/exclusion criteria. GH was found to be unusually polymorphic, with 26 SNPs in a span of 2,350 nucleotides. Most of these polymorphisms were clustered in the promoter region of the gene. Logistic regression identified association ($p=0.03$) between FGR and two C alleles in complete linkage disequilibrium located at 1 and +3 relative to the start of transcription. Separate ANOVA within normal and FGR classes demonstrated an average reduction in birth weight of 152g among normal birth weight and 97g among FGR newborns bearing C alleles at these two sites. These birth weight reduction were marginally significant ($p=0.057-0.086$) due primarily to small sample sizes. There are several biological explanations for how variation at either site 1 or +3 might affect the level of expression of GH. A survey of three microsatellites and three SNPs spanning the IGF-1 gene indicates that polymorphism in the region of intron 2 is associated with FGR. This finding is consistent with independent work in other laboratories.

Familiality of Rectal Cancer as Studied in the Utah Population Database. *J.S. Maul*¹, *L. Cannon-Albright*². 1) Division of Hematology/Oncology, University of Utah, Salt Lake City, UT; 2) Division of Medical Informatics University of Utah, Salt Lake City, Utah.

Background: The UPDB is a database linking genealogy for 2.5 million Utah individuals to 85,000 Utah cancer records. The familial nature of cancer has been well studied using this resource. Here, we investigate familiality for 2,733 individuals with rectal cancer. **Methods:** We estimated relative risk (RR) of rectal cancer, colorectal cancer (CRC), colon cancer (large intestine, excluding rectum), proximal colon cancer (proximal to the splenic flexure) and distal colon cancer (located in and distal to the splenic flexure, including rectum) in first and second degree relatives of rectal cancer cases using internal age- and site-specific rates of cancer. We considered all 2,733 rectal cancer cases and the subgroup of 222 early rectal cancers diagnosed <50yrs. **Results:** Data for first degree relatives is shown. In 1st degree relatives of rectal cancer cases, the RR for rectal cancer is 2.16 (95% CI 1.82 to 2.54), colorectal cancer 2.08 (CI 1.90 to 2.28), colon cancer 2.07 (CI 1.86 to 2.30), proximal colon cancer 1.86 (CI 1.57 to 2.19) and distal colon cancer 2.19 (CI 1.95 to 2.44). In 1st degree relatives of early rectal cancer cases, the RR for rectal cancer is 4.74 (CI 2.52 to 8.10), colorectal cancer 4.12 (CI 2.90 to 5.68), colon cancer 3.91 (CI 2.53 to 5.77), proximal colon cancer 3.39 (CI 1.63 to 6.24) and distal colon cancer 4.63 (CI 3.05 to 6.73). **Conclusions:** The UPDB provides strong evidence for a genetic component in rectal cancer, especially in early onset cases. Relatives of rectal cancer patients are at significantly increased risk of CRC in all segments of the large bowel and rectum. This risk is significantly higher in almost all segments of the colon than the increased risk for CRC among first degree relatives of CRC probands. Given a low frequency of genetic syndromes causing increased risk for rectal cancer, our findings suggest the presence of an unidentified genetic component for this disease. Multiple high risk rectal cancer pedigrees have been identified in the UPDB; study of these pedigrees could allow identification of rectal cancer predisposition genes.

Increasing power for tests of genetic association in the presence of phenotype and/or genotype error by use of double-sampling. *D. Gordon*¹, *Y. Yang*¹, *C. Haynes*¹, *S.J. Finch*², *N.R. Mendell*², *A.M. Brown*^{3,4}, *V. Haroutunian*⁵. 1) Statistical Genetics, Rockefeller University, New York, NY; 2) Applied Math and Statistics, Stony Brook University, Stony Brook, NY; 3) Burke Medical Research Institute, White Plains, NY; 4) Weill Medical College of Cornell University, New York, NY; 5) Bronx VA Medical Center, Bronx, NY.

We present a method, the likelihood ratio test allowing for errors (LRT_{ae}) that incorporates double-sample information for phenotypes and/or genotypes on a sub-sample of cases/controls. Population frequency parameters and misclassification probabilities are determined using Tenenbein's double-sample procedure (Tenenbein 1970; Tenenbein 1972) as implemented in the Expectation-Maximization (EM) method of Dempster et al. (Dempster et al. 1977). To compare our method with the standard method that makes no adjustment for errors (LRT_{std}), we perform null and power simulations assuming a SNP marker or a 4-allele marker locus, three different sets of cases/controls, two different settings each of phenotype and genotype error, and a 17% sub-sample of individuals double-sampled on either phenotype and genotype. We also apply our method to case/control ApoE genotype data for an actual Alzheimers study. The LRT_{ae} method maintains correct type I error proportions for all null simulations and all significance level thresholds (10%, 5%, 1%, 0.1%). LRT_{ae} power is always greater than or equal to LRT_{std} power. Power differences range from 0.0001 to 0.18 for the simulation parameters considered. LRT_{ae} average estimates of population frequencies and misclassification probabilities are equal to the true values, with variances of 10⁻⁷ to 10⁻⁸. For ApoE, the LRT_{ae} and LRT_{std} p-values are 5.8 x 10⁻⁵ and 1.6 x 10⁻³, respectively. The increase in significance is due to adjustment in the LRT_{ae} for misclassification of the risk allele. We also investigate cost/benefits of the two methods when budgets are fixed. We have developed freely available software that performs our LRT_{ae} statistic.

PARKIN Gene polymorphisms in Sporadic Parkinson's Disease. *M. Kasap¹, A. Sazci¹, E. Ergul¹, H. Tireli², M. Gencer², Y. Cetinkaya², G. Akpinar¹, G. Kaya³, I. Kara³.* 1) Department of Medical Biology and Genetics, Faculty of Medicine, University of Kocaeli, Kocaeli, Turkey; 2) Department of Neurology, Haydarpasa Numune Hospital, Uskudar, Istanbul; 3) Department of Neuroscience, Institute of Experimental Medical Research, University of Istanbul, Istanbul, Turkey.

Although the PARKIN gene was isolated from an autosomal recessive juvenile Parkinson patient, this gene has been reported to cause parkinsonism in sporadic late onset parkinson patients as well. Recently a polymorphism, S167N in exon 4 has been identified. We investigated the S167N polymorphism in 75 parkinson patients (36 women 48%; 39 men 52%), and 102 controls (50 women 49%; 52 men 51%). The PARKIN S167N polymorphism was amplified by PCR, followed by digestion with AlwNI restriction endonuclease. The size of the amplified fragment was 261 bp. When the fragment was digested with AlwNI, two fragments were created with a 167 bp, and 94 bp. The allele frequency of PARKIN 167N is 10% in the parkinson patients, and 5.39% in the controls. The frequencies of the PARKIN S167S, S167N, and N167N were 85.3%, 9.3%, and 5.3% in the parkinson patients and 90.2%, 8.8%, and 1.0% in the controls. Although the PARKIN N167N genotype showed an increased risk for parkinsonism in a Turkish population (OR=5.690; 95% CI= 0.623-51.986; Chi-2=2.983; df=1; P=0.084), the 95% CI range was rather wide.

Coding sequence variation in human sweet and umami taste receptor genes. *U. Kim, N. Riaz, D. Drayna.*
NIDCD/NIH, Rockville, MD.

Sweet and umami (the taste of glutamate) tastes play a major role on the perception of calorically rich and essential nutrients. In humans, three members of the T1R class of taste-specific G protein-coupled receptors (T1R1, T1R2, and T1R3), which reside on chromosome 1, are known to function in combination as heterodimeric receptors for sweet and umami tastes. We hypothesized single nucleotide polymorphisms (SNPs) or variant haplotypes of the T1R genes in humans may underlie individual differences in the perception and recognition threshold for sweeteners and amino acids. To enable study of genotype/phenotype correlations for these two tastes, we identified coding sequence variation by sequencing these genes in a cohort of unrelated individuals. To achieve maximum genetic diversity in our sample, we sequenced a panel consisting of 30 Europeans, 20 East Asians, 10 Native Americans, 8 South Asians, and 20 sub-Saharan Africans. In the combined sample, we found a total of 28 SNPs in these three genes. Sixty percent of these SNPs cause an amino acid substitution in the encoded receptor protein, and 1 SNP, in the T1R1 gene, introduces an in-frame stop codon. Although the size of these three genes is much larger than those of the T2R genes that encode bitter taste receptors, the number of cSNPs in the T1R genes is small compared to the number of cSNPs in T2R genes. This suggests that the sequence of the T1R receptor proteins are more conserved than those of T2R receptors, and may explain why individuals do not show broad variation in these taste modalities.

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Test of independence of highly polymorphic haplotype blocks. *H.S. Lee, R. Chakraborty.* Center for Genome Information, Dept. Environmental Health, Univ Cincinnati, Cincinnati, OH 45267.

Although haplotype blocks are generally demarcated by lack of linkage disequilibria between them, a formal test of independence between haplotypes of different blocks by the usual tests may lead to degeneracy, particularly when the number of possible haplotypes within blocks is large. We develop a new algorithm for testing whether or not haplotypes of different haploblocks are at linkage equilibrium. This test is based on pairwise comparisons of all composite haplotypes in a sample, where each haplotype comparison is scored in reference to the number of mismatched sites within each block. This generates a $(r + 1)$ by $(c + 1)$ contingency table, with r and c being the number of polymorphic sites defining the two haploblocks. Test of independence is then carried out with a chi-square or a likelihood ratio test statistic, for which the empirical level of significance is computed by permutation. This algorithm is applied to data on genotypes at 13 short tandem repeat (STR) loci and 12 Y-chromosome STR loci for 18 populations, the objective of which was to test that autosomal genotypes are independent of Y-chromosomal haplotype in absence of population substructure. This specific application also illustrates that such tests of independence between hypervariable haploblocks can be conducted with phase unknown genotype data for one or both haploblocks, where the number of mismatched sites is replaced by the number of alleles shared between multilocus genotypes. One novel population genetic inference that emerges from this test is that in pairs of individuals from a population of restricted effective size, the number of alleles shared is larger (and consequently, the number of mismatched sites is smaller) in general compared with those in large populations, but population size restriction alone do not necessarily induce linkage disequilibrium between haplotypes of distinct blocks. (Research supported by NIH grants GM 41399 and a training grant on research in bioterror agents).

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BioBank Japan for developing personalized medicine. *Y. Ohnishi*¹, *Y. Nakamura*^{1,2}. 1) SNP Research Ctr, RIKEN, Tokyo, Japan; 2) Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan.

Personalized medicine project started in June 2003 supported by Japanese government. The aims of this project are 1) discovery of genes susceptible to diseases, or those related efficacy or adverse reactions of drugs, 2) evidence-based development of drugs or diagnostic methods, 3) providing the important medical information that can be applied for establishment of Personalized Medicine and 4) studies on gene-environment interaction for prevention of diseases. To achieve these goals, we plan to collect of DNAs, sera and clinical information from 300,000 patients who mainly have common diseases by March 2007. For sample storage and clinical information database, we constructed BioBank Japan that consisted of facilities for DNAs and sera and established the computer system for clinical database. For protection of individual information, we are doing 1) two-step anonymization of individual identification code by 2D bar-code and 2) avoidance of placing individual identification information and genotyping information together. By the end of June in 2004, we have collected over 55,000 samples (a total number of disease cases is counted to be more than 70,000 due to multiple diseases in one patient) from 51 hospitals enrolled in this project. We have started systematic genomics analysis (the genome-wide association studies with 52,608 SNPs) to identify genes of medical importance using these samples. Accumulative data should provide us the basis to personalize the patients treatment.

Can we predict the risk of common complex diseases using multiple genetic tests? *C.M. Van Duijn¹, A.C.J.W. Janssens^{1,2}, Y.S. Aulchenko¹, S. Elefante¹, G.J.J.M. Borsboom², E.W. Steyerberg²*. 1) Epidemiology & Biostatistics, Erasmus University Medical Center, Rotterdam, Netherlands; 2) Department of Public Health, Erasmus University Medical Center, Rotterdam, Netherlands.

Complex diseases cannot be predicted with single genes, but there is ongoing debate on the future of multiple genetic tests. We evaluated the discriminative ability of tests including up to 400 susceptibility genes using the area under the ROC curves (AUC). We simulated three populations of 100,000 subjects in which the disease prevalence varied from 1%, 10% and 30% using R software. Allele frequencies of the risk allele varied from 10%-50% and odds ratios (ORs) from 1.25 to 3 (multiplicative effects). The number of genes involved and strength of their association with disease determined the AUC. When the frequency of disease and risk alleles are both 10%, at least 7 genes with strong effects (OR = 3.0) are needed in the tool to obtain an AUC of 0.80. When all ORs are 2.0, at least 18 genes are needed to obtain an AUC of 0.80, while 60 genes are needed when ORs are 1.5, and 170 when all ORs are 1.25. Also one can reach an AUC of 0.80 by combining a few strong genes with multiple weak genes, which is a more realistic scenario for most complex diseases. The maximum discriminative ability of a genetic screening tool could be deduced from the heritability and the prevalence of the disease. Based on our simulations studies it is unlikely that we will be able to predict common diseases with a modest heritability such as cardiovascular disease. However, predictions appear to be possible for diseases which have a higher heritability or are less common like Alzheimers disease and auto-immune diseases.

Molecular study of Alpha thalassemia in the multi-racial groups in Malaysia. *Y.C. Wee¹, J.A.M.A. Tan¹, K.L. Tan¹, W.P. Chow², S.F. Yap³.* 1) Department of Molecular Medicine, University Of Malaya, Kuala Lumpur, Malaysia; 2) Gleneagles Intan Medical Centre, Kuala Lumpur, Malaysia; 3) Department of Pathology, University of Malaya, Kuala Lumpur, Malaysia.

Thalassemia is the most common genetic blood disorder of hemoglobin synthesis in Malaysia. Alpha thalassemia is caused by deletions or mutations within the α -globin gene complex located on chromosome 16. Alpha thalassemia ($--^{SEA}$, $-^{3.7}$, $-^{4.2}$ and CS) was characterized in 670 pregnant women (MCV89 fL and/or MCH28 pg) who attended the Antenatal clinic of University Malaya Medical Centre (402 Malays, 100 Chinese, 148 Indians and 20 from other racial groups). 106 pregnant women (15.8%) were identified as α -thalassemia carriers - 3.7% (25) possess the $--^{SEA}$ deletion, 10% (67) were heterozygotes for the $-^{3.7}$ deletion, 0.6% (4) were heterozygotes for the $-^{4.2}$ deletion, 1.3% (9) were heterozygotes for Hemoglobin Constant Spring (Hb CS) and 0.2% (1) was compound heterozygous with the $-^{3.7}/^{CS}$ genotype. The $--^{SEA}$ deletion responsible for Hemoglobin Barts hydrops fetalis syndrome was found to be significantly higher (15%, $p < 0.0001$) in the Chinese, followed by a lower frequency in the Malays (2.5%). This double α -globin gene deletion was not detected in other racial groups. The $-^{3.7}/$ genotype was distributed evenly among the 3 racial groups - 10.7% of the Malays, 10% of the Chinese and 7.4% of the Indians. It was also detected in a woman with Thai nationality, an Arabic Malay woman with Yaman ancestry and in an Orang Asli (Aborigine) patient belonging to the Bidayuh tribe. The $-^{4.2}/$ genotype appeared to be at a significantly lower frequency (0.6%) as compared to the $-^{3.7}/$ genotype (10%, $p < 0.001$) and it was present only in 1% of the Malays. Only 1 Malay woman (0.15%) was found to be a compound heterozygote with the $-^{3.7}/^{CS}$ genotype and heterozygotes Hb CS ($^{CS}/$) was also detected only in the Malays (2.2%) in this study.

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QTL study of non-verbal mild mental impairment using DNA pooling and microarrays to screen over 10,000 SNPs. *L.M. Butcher, E. Meaburn, L. Schalkwyk, I.W. Craig, R. Plomin.* SGDP Centre, Institute of Psychiatry, London, United Kingdom.

Mild Mental Impairment (MMI) represents the quantitative low extreme of general intelligence and is highly heritable. Quantitative Trait Loci (QTLs) that confer susceptibility to MMI are of small effect size and are likely to be the same as those that operate throughout the distribution of intelligence, as well as other related traits. Achieving satisfactory power to detect such QTLs requires an association study design to screen many thousands of single nucleotide polymorphisms (SNPs) in large samples of individuals, preferably with minimal constraints on time and cost. To screen many thousands of SNPs we used the Affymetrix 10K Mapping GeneChip. To genotype large sample sizes we employed DNA pooling. In an experiment *a priori* which combined the Affymetrix 10K Mapping GeneChip and DNA pooling, we were able to show rapid estimation of allelic frequencies in DNA pools with a high degree of accuracy and reliability. We identified 515 MMI cases and 1,028 representative controls from a community-based longitudinal research project (Twins Early Development Study; TEDS) with data for over 14,000 7-year old children assessed on a diverse battery of cognitive tests. We genotyped DNA pools consisting of MMI cases and representative controls and compared relative allele frequencies for over 10,000 SNPs. In order to reduce false-positive marker selection for subsequent testing we also genotyped DNA pools consisting of the top (n=505) and bottom (n=503) extremes of the general intelligence distribution. For each group every individual was randomly assigned to one of five DNA sub-pools. Each sub-pool was genotyped on a separate microarray and allele frequency estimates were averaged across the five microarrays to provide a group estimate of allele frequency with sample variance. 36 SNPs showing differences .1 in both studies were individually genotyped on approximately 7500 individuals from the TEDS to test their association with MMI. This strategy provides a quick, accurate and economical way to screen thousands of SNPs in large samples and represents a genuine step forward in high-throughput genotyping in the hunt for QTLs of small effect size.

Properties of ranks associated with true positives in association genome scans. *D.V. Zaykin.* Genetic Data Sciences, GlaxoSmithKline, Inc, Res Triangle Park, NC.

Deciding which markers to type in further samples following an association scan across a genomic region or entire genome is difficult. The genotyping and analysis costs of following up a large number of signals must be balanced with ensuring true associations are among those selected for further investigation. Once a genome scan is completed the results can be sorted by the association statistic value. What is the probability that true positives will be encountered among the markers with the largest association statistic? This problem can be studied using the probability distribution of ranks allowing for dependence among associations due to LD. Using this setup, the effects of multiple testing corrections on the distribution of the true association ranks can be evaluated as well. This formulation allows to calculate the number of markers required to consider in a follow-up study in order to capture true associations with high probability. The results show that LD does not considerably affect the distribution of ranks. Further, it is found that the effect of multiple testing corrections on the distribution of ranks is generally weak. Furthermore, "multiple testing corrected"; results are likely to represent false positives, unless the power associated with true positives is very high. Association genome scans with 100K or more genetic polymorphisms have been evaluated. The results suggest that it is typical for genuine strong associations to rank among first hundreds or even thousands of results sorted by the value of the association test statistic. Therefore following up on too few markers can result in missing the true associations. Finally, the required sample sizes can be calculated to ensure that genuine associations are encountered among the "first runners".

Genome-wide Admixture Mapping for Hypertension Loci: The NHLBI Family Blood Pressure Program. *X. Zhu¹, A. Luke¹, R.S. Cooper¹, T. Quertermous², C. Hanis³, T. Mosley⁴, C.C. Gu⁵, H. Tang⁶, D.C. Rao⁵, N. Risch^{7,8}, A. Weder⁹.* 1) Preventive Med & Epidemiology, Loyola Univ Medical Ctr, Maywood, IL; 2) Division of Cardiovascular Medicine, Stanford Univ School of Medicine, Stanford, CA; 3) Dept of Genet, Univ of Texas, Houston, TX; 4) Dept of Medicine, Univ of Mississippi, Jackson, MS; 5) Division of Biostatistics, Washington Univ, St. Louis, MO; 6) Division of Public Health, Fred Hutchinson Cancer Research Center, Seattle, WA; 7) Dept. of Genetics, Stanford Univ, Stanford, CA; 8) Division of Research, Kaiser Permanente, Oakland, CA; 9) Dept of Medicine, Univ. of Michigan, Ann Arbor, MI.

Discovery of genetic variants that contribute to risk of hypertension has proven to be a challenge. As a complement to linkage and candidate gene association studies, we performed genome-wide admixture mapping using microsatellite markers among the African-American participants of the NHLBI Family Blood Pressure Program (FBPP). The population sample was assumed to have experienced recent admixture from ancestral groups originating in Africa and Europe. A set of unrelated individuals from Nigeria was used to represent the African source population, while the whites in the FBPP provided estimates for Europe. Two hundred and sixty nine microsatellite markers were typed in common in the three groups at the same laboratory. The distribution of marker-specific African ancestry was shifted upward in hypertensive cases versus normotensive controls, consistent with linkage to genes conferring susceptibility. This shift was largely due to a small number of loci, including five adjacent markers on chromosome 6q and two on chromosome 21q. The results indicated that chromosome 6q24 and 21q21 harbor genes influencing risk of hypertension in African Americans.

An effective screening of informative SNP by restriction fragment mass spectrometry(RFMS). *S. Hwang¹, S.P. Hong², H.B. Oh¹.* 1) Laboratory Medicine, Asan Medical Center, Seoul, Korea; 2) GeneMatrix Inc., Seoul, Korea.

Introduction: Despite the completeness of current SNP databases, it was recently reported that almost a third of all SNP in dbSNP (www.ncbi.nlm.nih.gov/SNP) are population-specific or non-informative with a minor-allele frequency less than 10%. Thus, selecting informative SNP from public databases rather than detecting new SNP becomes an important initial step. One efficient way is DNA pooling, where allele frequencies are examined in a small number of pools rather than a large number of individuals. In this report, we applied RFMS to screen pooled DNA which is based on matrix-assisted laser desorption/ionization time-of-flight technique. **Method:** One hundred thirty-nine coding non-synonymous SNPs (cnSNP) of thirty asthma-related genes were selected from dbSNP (www.ncbi.nlm.nih.gov/SNP). The pooled DNA from 200 samples was PCR-amplified and digested with FokI and BstF5I enzymes. The restricted fragments were analyzed on a Linear MALDI-TOF MS (Bruker Daltonics Biflex IV, USA) workstation. Each 10 individual samples of patient and normal groups were genotyped to validate the results. **Result:** It was found that only thirteen of 139 selected cnSNPs (9.4%) were informative with a minor-allele frequency more than 10%;(table) in Koreans. **Conclusion:** RFMS is a very useful method in selecting informative SNPs. The restricted masses are well separated as dual peaks and thus easy to read whether the SNP is heterozygous or not. It is highly recommended that if you are planning to study with cnSNPs, selection of informative SNPs by a method as we did be preceded before a large-scale association study.

gene locus	FGFR4	FGFR4	KDR	KDR	TLR3	IL4R	IL4R	MDR1	MDR1	MDR1	IL12RB1	IL12RB1	CCR2
rs #	rs196626	rs351855	rs1870377	rs2305948	rs377529	rs1805010	rs1801275	rs1045642	rs2032582	rs2707944	rs401502	rs375947	rs1799864
allele frequencies (n=20)	A 0.55/G 0.45	C 0.59/T 0.41	A 0.48/T 0.52	C 0.90/T 0.10	G 0.70/A 0.30	A 0.75/G 0.25	A 0.75/G 0.25	C 0.58/T 0.42	A 0.25/G 0.30/T 0.45	C 0.50/T 0.50	C 0.60/G 0.40	A 0.70/G 0.30	A 0.30/G 0.60

Detection of SNPs haplotypes by a Non-fluorescent multiplex DHPLC method. *S. Korem¹, V. Meiner², C. Shocaa¹, E. Leitersdorf³, D. Bercovich¹.* 1) Dept of HMG & Pharmacogenetics, MIGAL- Galilee Bioechnology Center, Kiryat Shmona; 2) Dept of Human Genetics, Hadassah-University Hospital, Jerusalem; 3) Dept of Medicine B, Hadassah-University Hospital, Jerusalem, Israel.

Over the past few decades, different approaches have been developed to identify genetic alterations. Existing procedures for the detection of unknown point mutations, has its advantages and limitations, but the technical aspects and/or cost of the operations have not made their use routine in most laboratories. Further developments of highly accurate, rapid, inexpensive, automated and easily interpreted techniques are required. Denaturing high-performance liquid chromatography (DHPLC) is a sensitive, robust and operationally inexpensive method in comparison to direct sequencing for the detection of unknown single-base substitutions and small deletions and insertions. Analysis of one fragment can take up to 5-6 min. To increase sample throughput, we have developed a non-fluorescent multiplexing strategy for the analysis of three different amplicons, using only one gradient and temperature, by identifying the most limiting parameters for distinguishing between heteroduplex and homoduplex PCR products when they are in a multiplex state. The Wavemaker or Meltdoc software routinely deduces the gradient and temperature of a given PCR amplicon, but in combining amplicons in one run, there is no guideline for which one temperature or gradient will be the most adequate for multiplexing DHPLC. Haplotypes rather than a single SNP are associated with the degree of response to the lipid-lowering therapy. We developed a multiplex DHPLC method for detecting SNPs and apply it to the Cholesteryl-ester transfer protein (CETP) and P-glycoprotein drug transporter (MDR1) genes, which are of important interest in hypercholesterolemia therapy with Statin drugs. By analyzing SNPs from the MDR1 (very AT rich) and CETP (GC rich) genes, we show that it is possible to use the same conditions for three different amplicons by designing them in appropriate sizes, by choosing the higher TM from all three fragments and the gradient percentage of the longest fragment. This technique can be adopted for GC rich and AT rich fragments.

Inbreeding coefficient estimation using only the individuals' genomic information in the Hutterite population. A. Leutenegger^{1,2}, C. Bourgain¹, C. Ober³, F. Clerget-Darpoux¹. 1) U535, INSERM, Villejuif, France; 2) U289, INSERM, Paris, France; 3) Dept Human Genetics, University of Chicago, Chicago, IL.

We have recently proposed a maximum likelihood method (Leutenegger et al, 2003) to estimate the inbreeding coefficient of an individual (the probability that two alleles at a locus are identical by descent (IBD)) using only his genomic information. From simulations, we found that we could obtain accurate estimates whenever the marker map was dense relative to the average IBD segment length expected from the genealogy. Here we further study the properties of this estimator on real data from a well characterized population isolate, the Hutterites of South Dakota. In this population, individuals derive from 64 founders living in the 18th century and are related through multiple lines of descent. Extensive pedigree information is available. We look at a large sample of > 600 individuals who have been genotyped for a dense microsatellite map (658 markers, ~5cM spacing, average het.=0.7). All the individuals are inbred: the average inbreeding coefficient estimated using the genealogical information is 0.034 with 95% of the estimates contained in [0.012, 0.059]. The correlation between our estimate and the one expected from the genealogy is not very high. This has probably two reasons. One is, as we have shown before, that the true proportion of genome IBD (which our estimator reflects) may be far from the proportion expected from the genealogy. The other one is the structure of the Hutterite pedigree: for a given inbreeding coefficient, a Hutterite individual will tend to have multiple remote common ancestors rather than a unique and more recent one. Therefore, the expected IBD segment length will tend to be smaller than in a simple pedigree and a denser map will be needed to catch the homozygous markers. Using simulations on a few individuals, we will propose a map density necessary to obtain an accurate estimation of the inbreeding coefficient with our method.

Association of Single-Nucleotide Polymorphisms with Inflammation and Risk of Cardiovascular Disease in a Dialysis Cohort. *Y. Berthier-Schaad*^{1,2}, *Y. Liu*¹, *L.C. Plantinga*¹, *N.E. Fink*¹, *M.J. Klag*¹, *J. Coresh*¹, *M.W. Smith*³. 1) Johns Hopkins Medical Institutions, Baltimore, MD; 2) Laboratory of Genomic Diversity, NCI, Frederick, MD; 3) Basic Research Program, SAIC, NCI, Frederick, MD.

Genetic analysis gives the opportunity to test the importance of inflammation in cardiovascular disease (CVD) because of a clear causative pathway. Genes from inflammatory pathways were chosen for analysis with special inclusion of those previously reported as associated with CVD. We genotyped 63 SNPs from 14 genes, in the CHOICE cohort of 775 [529 European Americans (EA), 246 African Americans (AA)] incident dialysis patients. Genotyping was done using TaqMan and haplotypes were estimated using PHASE. Linear and Cox regression were used to estimate the mean difference in inflammatory marker (IL-6, CRP, and albumin) levels and risk of CVD events, respectively, by genotype and haplotype. Adjustment variables included age, sex, race, dialysis modality, smoking, and comorbidities, plus blood pressure, HDL, and cholesterol for the risk models. Significant associations of several functional variants with CVD risk were found in IL6, LTA and TGFB1: the first two were also related to inflammation measures. Four genes showed inconsistent associations. One FGB allele (-455 A) was marginally associated with increased fibrinogen levels. One IL10 allele (-1082G) was associated with increased CRP levels in EAs, but there were no other consistent associations with inflammation or CVD. The slightly protective effect of the common PPARA haplotype in AAs was in the opposite direction of previous associations reported in an European population. IFNG -179T present only in AAs was associated with lower inflammation marker levels but not with decreased CVD mortality. In ACE, IL4, PPARG, ABCG5/G8, GNB3, and HBB (sickle cell), no associations were found between genotypes or haplotypes and inflammation marker levels or CVD risk after adjustment. Significant associations with three genes, IL6, LTA and TGFB1, support a causative role of inflammation in cardiovascular disease. Funded in part by RO1 L62985, AHA EIO140197ND, &DHHS#NO1-CO12400.

CAP70 as a possible modifier gene of Cystic Fibrosis phenotype. *S. Gambardella¹, M.R. D'Apice¹, C. Bulli¹, P. Borgiani¹, S. Russo¹, V. Lucidi², G. Castaldo³, G. Novelli¹.* 1) Biopathology , Tor Vergata University, Roma, RM, Italy; 2) Pediatric Hospital "Bambino Gesù" Rome, RM, Italy; 3) Department of Biochemistry and Medical Biotechnology, University of Naples "Federico II" and CEINGE-Biotecnologie Avanzate, Naples, Italy.

Cystic fibrosis (CF; #219700) is an autosomal recessive disorder caused by mutations in the CFTR gene that encodes a chloride conducting channel expressed in the apical plasma membrane of the epithelial cells. The disease is characterized by a wide variability of clinical expressions (pancreatic insufficiency, lung disease, hepatic manifestations, male infertility, etc.). It is possible that the wide phenotypic spectrum is modulated by modifier genes which directly or indirectly interact with CFTR gene product. Recently, biochemical and functional analysis of CFTR, revealed that this protein interacts with protein containing multiple PDZ domain (Cftr Associated Protein 70 kDa, N/H Exchanger Regulatory Factor). We studied a human homolog of the mouse PDZK1, CAP70, as a candidate CF modifier gene. CAP70 maps on 1q21 and is organized in 8 exons which encode a mRNA of 2.2Kb. The gene presents three actively transcribed copies, but one of these is translated in a functional protein product. We genotyped CAP70 in a cohort of CF patients with pancreatic insufficiency (PI=36), hepatic involvement (EI=31), and classical CF phenotype (CFC=70). Results were compared with a matched age normal controls (C=35). Genotypes at SNP IVS4 92 A/G (rs9726178) upstream of the exon 5 were examined by Pyrosequencing technology to determine the allele copies in a triplicate gene assay. This method is able to reveal seven different allele combinations (6A0G; 5A1G; 4A2G; 3A3G; 2A4G; 1A5G; 0A6G). The allelic distribution was different within CF phenotypic classes compared (Chi square test: $p=0.012$). In particular, 3A3G were underrepresented within the PI CF patients ($p=0.008$), while 5A1G, 6A0G, 0A6G, 1A5G were overrepresented in this group of patients ($p=0.007$). These data strongly suggest a role for CAP70 gene as modifier of the CF phenotype.

Comparing analytic strategies for genome wide association using real data. *K. Klos¹, A. Rosin¹, A.-H. Maitland-van der Zee¹, A.C. Morrison¹, T. Woodage², A. Litvinenko¹, E. Boerwinkle¹.* 1) Human Genetics Center, University of Texas Health Science Center, Houston, TX; 2) Applied Biosystems Inc., Foster City, CA.

We have carried out a pilot study of genome wide association for a common chronic disease using approximately 3000 SNPs spanning human chromosome 19 typed in approximately 2000 individuals. The overall goal of the study is to identify genes contributing to disease risk. For this pilot study, we compared contemporary statistical and computer science-based methods for association analyses, keeping in mind the overall goal. We observed significant concordance in the number of SNPs selected for developing a best model for predicting disease occurrence. For example, both the machine learning classification algorithms (e.g. random forests, AdaBoost) and the set association approach of Hoh and Ott selected approximately 350 SNPs as the upper bound for the dimension of the model building step. Within this set of 350 SNPs, there was also significant concordance in the identity of the selected SNPs. The rank correlation within the top 350 SNPs was 0.45 or greater between statistical and machine learning-derived metrics. There was minimal concordance between genes selected based on single site analyses and genes selected by haplotype analysis (e.g. $r=0.15$). We conclude that a hierarchical approach beginning with data exploration, then model building using SNPs or haplotypes, and then model validation is most appropriate for genome wide association studies. We have projected the computational burden of such a hierarchical approach to a data set containing up to 300,000 SNPs and find it to be scalable within the computing resources of most investigators.

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Inference on Haplotype-Disease Associations in DNA Pooling Studies. *D.Y. Lin, D. Zeng.* Dept Biostatistics, Univ North Carolina, Chapel Hill, NC.

The genetic dissection of complex human diseases requires large-scale association studies which explore the population associations between genetic variants and disease phenotypes. DNA pooling can substantially reduce the cost of genotyping assays in these studies and thus enables one to examine a large number of genetic variants on a large number of subjects. The availability of pooled genotype data instead of individual data poses considerable challenges in the statistical inference, especially in the haplotype-based analysis because of increased phase uncertainty. We present a general likelihood-based approach to making inference about haplotype-disease associations based on pooled DNA data. We consider cohort and case-control studies of unrelated subjects, and allow arbitrary and unequal pool sizes. The phenotype can be discrete or continuous, univariate or multivariate. The effects of haplotypes on disease phenotypes are formulated through flexible regression models, which allow a variety of genetic hypotheses and gene-environment interactions. We construct appropriate likelihood functions for various designs and phenotypes, accommodating Hardy-Weinberg disequilibrium. The corresponding maximum likelihood estimators are approximately unbiased, normally distributed and statistically efficient. We develop simple and efficient numerical algorithms for calculating the maximum likelihood estimators and their variances, and implement these algorithms in a freely available computer program. We assess the performance of the proposed methods through simulation studies, and provide an application to the Finland-United States Investigation of NIDDM Genetics Study. The results show that DNA pooling is highly efficient in studying haplotype-disease associations.

Haplotype Diversity in the CCL18, CCL3, and CCL4 chemokine genes is Associated with HIV-1 transmission and AIDS disease progression. *W.S. Modi¹, J. Lautenberger², K. Scott⁸, A. Ping⁸, J.J. Goedert², S. Strathdee³, S. Buchbinder⁴, J. Phair⁵, S. Donfield⁶, S. O'Brien⁷, C. Winkler⁸.* 1) SAIC, NCI-ATC, Gaithersburg, MD; 2) Viral Epidemiology Branch, National Cancer Institute, Rockville, MD 20852, USA; 3) Johns Hopkins School of Hygiene and Public Health for AIDS Link to the Intravenous Experience, Baltimore, MD 21205, USA; 4) San Francisco Department of Public Health, San Francisco, CA 94102; 5) Department of Medicine, Northwestern University Medical School, Evanston, IL 60611; 6) Rho, Inc., Chapel Hill, NC 27514, for Hemophilia Growth and Development Study; 7) Laboratory of Genomic Diversity, National Cancer Institute-FCRDC, Frederick, MD 21702-1201; 8) SAIC Frederick, National Cancer Institute-FCRDC, Frederick, MD 21702-1201.

CCL3, CCL4, CCL18 are potent chemoattractants produced by macrophages, natural killer cells, fibroblasts, mast cells, CD4+ and CD8+ T-cells. CCL3 and CCL4 are natural ligands for the primary HIV-1 coreceptor CCR5. Further, CCL3 and CCL4 are known to activate and enhance the cytotoxicity of natural killer cells. Genomic DNAs from 3000 participants enrolled in five USA-based natural history AIDS cohorts were genotyped for 21 single nucleotide polymorphisms (SNPs) in the a 46 kb interval containing these genes. Seven of the 21 SNPs had minor allele frequencies less than 0.5% in European-Americans, while all 21 were variable in African-Americans. Four or eight haplotype blocks were observed in European-Americans or African-Americans, respectively. Blocks were strongly correlated with each other and haplotype diversity within blocks was limited, indicating strong linkage disequilibrium across the entire region. Frequencies of three SNPs and their haplotype in the CCL3 gene were elevated in frequency ($p=0.04$ to 0.09) among highly exposed, persistently seronegative African Americans compared to seroconvertors. Nine highly correlated SNPs spanning the CCL3 and CCL4 genes were significantly associated ($p=0.0008$ to 0.05) with more rapid disease progression in European Americans. These results reiterate the importance of chemokine gene variation in HIV-1/AIDS pathogenesis.

Heritability of Iron and Ferritin in a genetic isolate. *O.T. Njajou¹, B.Z. Alizadeh¹, B.A. Oostra¹, D. Swinkels², C.M. van Duijn¹.* 1) Epidemiology & Biostatistics, Erasmus University Medical Centre, Rotterdam, The Netherlands; 2) Clinical Chemistry, University Medical Centre, Nijmegen, The Netherlands.

BACKGROUND: Iron plays a crucial role in the pathogenesis of common complex disorders such as Parkinson's disease, Alzheimer's disease, atherosclerosis and cancer. Iron deficiency affects about 30% of the world's population and genetic iron overload or hemochromatosis is the most common inherited disorder in Caucasians. Both iron deficiency and iron overload are common public health problems. From a genetic perspective, iron metabolism is a complex trait, in which both genetic and environmental factors (nutrition) are involved. **AIM:** The purpose of the present study was to estimate the magnitude of genetic influences on iron and ferritin levels in nuclear families derived from a genetic isolate in the Netherlands (the ERF study). **METHOD:** Estimation of how much of the variation in the levels of iron and ferritin could be attributed to genetic factors was done using the variance component method in sequential oligo-genic linkage analysis routines (SOLAR). **RESULTS:** The ERF participants analysed in this study included 90 nuclear families with a total of 908 subjects. The proportion of the residual phenotypic variance due to additive genetic effects or heritability (h^2) estimates were approximately 21.34 (SE = 0.064, P 0.000001) for iron and 38.62 (SE = 0.066, P 0.000001) for ferritin. After simultaneously adjusting for sex and age, the heritability was 23.15 (SE = 0.064, P 0.000001) for iron and 39.20 (SE = 0.066, P 0.000001) for ferritin. Sex and age explained less than 5% variability in iron and ferritin level. **CONCLUSION:** A substantial proportion of the variance of iron and ferritin can be explained by heredity, independent of the effects of sex and age. These results demonstrate the influence of both genetic and environmental factors on iron levels. Identification of genes influencing iron and ferritin levels using a QTL approach is feasible.

Nonparametric Test of Association of Multiple Genes with Human Traits. *D.J. Schaid¹, S.K. McDonnell¹, S.N. Thibodeau²*. 1) Dept. Health Sciences Research, Mayo Clinic College of Medicine, Rochester, MN; 2) Dept. Laboratory Medicine and Pathology, Mayo Clinic of College Medicine, Rochester, MN.

Genetically complex traits are likely caused by multiple genes, each with small to moderate effects. Although it is now common practice to measure multiple genes by multiple markers, such as single nucleotide polymorphisms (SNPs), current statistical methods to analyze many markers are limited with weak power. For example, it is common to analyze the marginal effects of one marker at a time, or perhaps in a step-wise fashion with step-wise regression methods, or analyze all markers simultaneously with a global test that has many degrees-of-freedom. If many of the markers are causative, or in linkage disequilibrium with causative variants, yet the marginal association of each marker is weak due to low penetrance and possibly complex interactions among the causative variants, then the usual analytic methods will have weak power. This may be particularly hazardous when evaluating biologically related genes, such as genes within a common functional pathway. We developed a nonparametric statistic based on the pair-wise similarity of subjects according to their measured genotypes across all markers, and compares the average of this similarity between cases versus controls, which is expected to differ if the markers are associated with disease. Our methods account for missing marker data and correlations among the markers (i.e., linkage disequilibrium), they do not require assumptions of Hardy-Weinberg genotype proportions, and they weight the genetic markers according to their covariance structure, in order to create an optimally weighted statistic. The statistic has only one degree-of-freedom, which can give greater power than other global statistics. These new methods are applied to a large case-control study whose aim is to evaluate the association of prostate cancer with common genetic polymorphisms for enzymes within biosynthesis pathways that catalyze the synthesis and bioactivation of androgen and estrogen. In addition, simulations are used to evaluate the power and utility of these new methods.

The heritability of mammographically dense and non-dense breast tissue. *J. Stone*¹, *G.S. Dite*¹, *A. Gunasekara*², *D.R. English*⁴, *M.R.E. McCredie*⁵, *G.G. Giles*⁴, *J.N. Cawson*⁶, *R.A. Hegele*⁸, *A. Chiarelli*⁷, *M.J. Yaffe*³, *N.F. Boyd*², *J.L. Hopper*¹. 1) Centre for Genetic Epidemiology, University of Melbourne, Australia; 2) Division of Epidemiology and Statistics, Ontario Cancer Institute, Canada; 3) Imaging Research, Sunnybrook and Women's College Hospital, Canada; 4) Centre for Cancer Epidemiology, The Cancer Council Victoria, Australia; 5) Department of Preventive and Social Medicine, University of Otago, New Zealand; 6) St. Vincent's BreastScreen, St. Vincent's Hospital, Australia; 7) Cancer Care Ontario, Canada; 8) Blackburn Cardiovascular Genetics Laboratory, Robarts Research Institute, Canada.

Background: Percent mammographic density (PMD) is a strong risk factor for breast cancer. Our previous twin study showed that the heritability of PMD was about 60%. The purpose of this paper is to determine the heritabilities of the components of PMD, the areas of dense and non-dense tissue. *Methods:* We combined two twin studies comprised of 571 monozygous (MZ) and 380 dizygous (DZ) twin pairs recruited from Australia and North America. Dense and non-dense areas were measured using a computer-assisted method, and information about potential determinants obtained by questionnaire. We estimated the heritability of the log dense area and log non-dense area, and the genetic and environmental contributions to the covariance between the two traits, using maximum likelihood theory. *Results:* After adjusting for measured determinants, for each of the log dense area and the log non-dense area, the MZ correlations were greater than the DZ correlations. Heritability was estimated to be 65% (95% confidence interval (CI) 60-70%) for dense area and 66% (95% CI 61-71%) for non-dense area. The correlations (s.e.) between the two adjusted traits were -0.35 (0.023) in the same individual, -0.26 (0.026) across MZ pairs and 0.14 (0.034) across DZ pairs. *Conclusion:* Genetic factors appear to play a large role in explaining variation in the mammographic areas of both dense and non-dense tissue. About two-thirds of the negative correlation is explained by the same genetic factors influencing both traits, but in opposite directions.

Relating the ApoE locus to unobserved loci using novel association statistics with respect to the Alzheimer phenotype. *S. Boehringer*¹, *E. Nagel*², *E.R. Martin*³, *A. Steland*². 1) Dept Humangenetik, Univ Duisburg-Essen, Essen, Germany; 2) Lehrstuhl für Stochastik, Ruhr-Universität Bochum, Bochum, Germany; 3) Center for Human Genetics, Duke University Medical Center, Durham, NC, United States.

A major challenge in genetic studies of complex traits is the characterization of small genetic effects at potentially many loci. It is commonly held that genetic heterogeneity is one major obstacle in the dissection of complex diseases or phenotypes. Therefore it is desirable to distinguish effects of observed regions of association from those of unobserved loci. We propose a method to estimate haplotype frequencies that include the causative allele in a region of association in a family based setting, i.e. linkage disequilibrium (LD) of observed haplotypes with a potentially unobserved causal locus can be reconstructed. Simultaneously the effect of unobserved loci is modelled with a small set of parameters. These parameters contain information about the phenocopy rate excluding the associated region under scrutiny. Further applications of the method include selection of individuals in resequencing efforts or the evaluation of the causativeness of variants. Since several assumptions are made to establish the likelihood framework we briefly show simulation results evaluating the robustness of the method. Alzheimer's disease is one of few examples where a predisposing causal effect of an allele (ApoE ϵ ₄) could be replicated in numerous samples. We apply the proposed statistical procedures to a data set of Alzheimer's disease containing nuclear families (Martin et al., AJHG 2000) which confirms the causative effect of the ApoE locus.

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Fine mapping of disease in case-control studies using haplotype-similarity measures. *M.P. Epstein*¹, *G.A. Satten*².
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A popular strategy for fine mapping a disease locus uses the idea that haplotypes from affected individuals should share longer stretches of identical sequence (and hence have increased similarity) around a disease locus relative to haplotypes from unaffected individuals. For a case-control study design, one can fine map a mutation by assessing whether the average similarity of haplotype pairs around a locus in a sample of case subjects is significantly greater than the average haplotype similarity in a sample of control subjects. Here, we develop likelihood-based score statistics for fine mapping that are based on measures of haplotype similarity between pairs of haplotypes in the case and control samples. We develop both retrospective and prospective versions of these score statistics and evaluate their performance using simulated data. We also apply both score statistics to real case-control data from the Finland-United States Investigation of Non-Insulin-Dependent Diabetes Mellitus (FUSION) Genetics study.

Association of 5HT2C SNPs in binge-eating purging anorexia, bulimia nervosa, and related psychopathological traits of eating disorders. X. Estivill^{1, 2}, M. Ribases¹, JM. Mercader¹, M. Gratacos¹, A. Badia³, L. Jimenez³, R. Solano³, F. Fernandez-Aranda³. 1) Genes and Disease Program, Centre for Genomic Regulation; 2) Experimental and Health Sciences Department, Pompeu Fabra University; 3) Hospital Princeps d'Espanya, Barcelona, Catalonia, Spain.

Several lines of investigation suggest that the serotonergic system participates in eating behavior and weight regulation. Moreover, alterations in this neurotransmitter system could participate in the etiology of eating disorders (ED) and in some associated psychopathological traits. To test this hypothesis, we analyzed the potential involvement of the -995G>A, -759C>T, -697G>A and the Cys23Ser SNPs within the serotonin receptor 5HT2C gene in a total sample of 151 ED patients (75 with anorexia (AN) and 76 with bulimia (BN)) and 116 sex-matched unrelated controls. The three promoter polymorphisms were in linkage disequilibrium and no association was observed when all SNPs were independently considered. However, the GCCC haplotype showed significant association with both binge-eating/purging AN (ANP; $p=0.03$) and BN ($p=0.017$). When we considered those patients showing purging attitudes we observed that the GCCC haplotype was positive associated to the purging phenotype ($p=0.01$). We then analyze the possible involvement of these sequence variants in different psychopathological traits measured with the Symptom Checklist 90-revised (SCL90-R) questionnaire. Forty-six patients with AN and 36 with BN completed the Symptom Checklist 90-revised (SCL90-R) questionnaire and were genotyped for the four SNPs within the 5HT2C gene. No effect of the Cys23Ser SNP was detected, but BN patients carrying the -995A/-759T/-967C haplotype showed increased symptomatology for seven of the nine subscales of the SCL90R, that include somatization ($p=0.029$), obsessive-compulsiveness ($p=0.021$), depression ($p=0.032$), anxiety ($p=0.004$), hostility ($p=0.028$), phobic anxiety ($p=0.029$) and paranoid ideation ($p=0.008$). These results suggest that the 5HT2C gene could participate in the physiopathology of ANP and BN through its direct effect on eating behavior and in different psychiatric symptoms related to the anxiety and depressive traits.

A general framework for estimating haplotypes & haplotype frequencies. *K.A.B. Goddard, D. Baechle.*
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Estimation of haplotypes & haplotype frequencies when phase is ambiguous is useful in a variety of situations including fine-mapping in family studies, and tests of association to detect candidate genes. Numerous methods are available to perform these functions that differ in the type of data (e.g., unrelated individuals, pools of individuals, and related individuals), and the statistical approach used to obtain the estimates, such as maximum likelihood (e.g., the EM algorithm), or Bayesian methods. We propose a general framework to incorporate four types of data into a common haplotyping procedure including unphased genotypes for unrelated individuals with or without partial molecular phase, marker allele frequencies for pools of unrelated individuals, and unphased genotypes for related individuals. Combinations of these types of data can be considered simultaneously. We have implemented this procedure, and utilize the EM algorithm to estimate haplotype frequencies once the data is in the common format. However, any statistical approach that can accommodate pooled data could be used. This procedure will provide haplotype frequency estimates, all possible haplotypes for each unit (i.e., individual or pool), and the most likely haplotypes for each unit.

We demonstrate the performance of this method for pedigrees and unrelated individuals using simulation. Estimated haplotypes & haplotype frequencies are compared to the true model parameters, and to the actual simulated sample using measures of information content. Incorporating partial phase information, obtained either from molecular haplotyping or through family data, into the estimation procedure improves the estimates of the haplotypes & haplotype frequencies. In addition, we discuss the practical limitations of this method in terms of the sample size, number of markers, and pedigree size that can be considered. The ability to utilize a common procedure for estimating haplotypes & haplotype frequencies under a broad range of circumstances will simplify haplotyping efforts, and will enable researchers to combine different types of data into a single estimation procedure.

Founder effect for the D157N mutation of the *MATP* gene with OCA4 from Japan and Korea. *K. Inagaki, T. Suzuki, S. Ito, N. Suzuki, Y. Tomita.* Dermatology, Nagoya Univ., Nagoya, Japan.

Oculocutaneous albinism type 4 (OCA4) was identified a new form of human OCA in 2001 that was a group of autosomal recessive hypopigmentary disorders in the skin, hair and eyes. The gene responsible for OCA4 is the *MATP* (Membrane-Associated Transporter Protein) gene located in chromosome segment 5p. The encoded protein, MATP is predicted to span the membrane 12 times on melanosome and likely functions as a transporter that mediates melanin synthesis. Previously, we identified 18 Japanese patients with OCA4; they harbored seven mutations (P58S, D157N, G188V, V507L, S90CGGCCA>GC, V144insAAGT and V469delG). One of them, the D(GAC)157N(AAC) mutation, was detected in 12 out of 18 Japanese patients, among whom 2 were homozygous and 10 were heterozygous. The D157N mutation allele was the most frequent; the allele frequency was 0.389 (14/36). In addition, we recently diagnosed a single Korean patient with OCA4, who was homozygous for the D157N mutation. In order to study the origin of the D157N mutation, we did haplotype analysis by means of three SNPs spanning the *MATP* gene and the flanking SNP. The 13 out of 14 alleles carrying the D157N mutation of Japanese patients shared a common haplotype, whereas the frequency of this haplotype in the Japanese control alleles (n=208) was estimated to be 0.13 using the Estimating Haplotype-frequencies (EH) software program. Statistical significance of the association was demonstrated using the Fishers exact test ($P<0.001$). The Korean patient shares this common haplotype, too. Until now, one Turkish and five German patients with OCA4 have been only reported in Western countries; they harbored one and ten mutations, respectively. However, the D157N mutation was not found in either. And furthermore, a common haplotype with the D157N mutation had the polymorphism E(GAG)272K(AAG) showed only in the Japanese and New Guinea Islander populations, but didn't have the polymorphism L(TTG)374F(TTC) existed exclusively in Caucasians. Here, we indicate that the D157N mutation of the *MATP* gene with OCA4 from Japan and Korea was a founder mutation and occurred after the divergence of East Asians and Caucasians.

Variability of haplotype-block patterns across populations: qualitative and quantitative differences. F.

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Our best hypothesis of Linkage Disequilibrium (LD) in humans is described by a block-like pattern, where SNPs show a high pairwise LD in well-defined regions of variable size. These blocks are interspersed with shorter areas of low or no LD. SNPs in blocks create haplotypes with limited variability, making a haplotype map-based LD approach to find genes for complex traits a feasible strategy. In fact, we can select and genotype only those SNPs that best identify the block haplotypes (htSNPs) without appreciable loss of information, but with a considerable reduction of costs. However, several issues remain to clarify before considering a haplo-block approach the best strategy to map genes. We do not yet know the proportion of genome characterized by a block pattern, nor the best number and density of SNPs needed for a complete information of LD. Also we do not know the degree of variability in LD and in haplotypes across populations. In fact, since LD is also a consequence of population evolution, we do not know if a block pattern found in a given population is generalizable to other populations: the data available today mostly derive from Gabriel et al (2002) who showed the existence of a similar pattern in macro populations, with Asians and Caucasians both being different from Africans that had a mean shorter block structure. We studied 10 different haplo-block regions in 5 Caucasian populations and found that there are either qualitative and quantitative variations. Quantitative variability implies that similar haplotypes are present in all populations despite at different frequencies, with a low influence on mapping, if any. However qualitative variations result in structurally different blocks, with various size and hence SNPs composition. These qualitative variations probably emerge because of putative recombination events or because boundary SNPs can vary: in either case, they affect mapping tasks and we present a method to (1) evaluate these differences and (2) to take them into account in mapping, especially when looking at more than one population.

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Candidate Gene Analysis of Cleft Lip and Palate in Japanese population. *J. Machida, Y. Suzuki, S. Suzuki, N. Matsuzawa, H. Furukawa, T. Niimi, N. Natsume, K. Shimozato.* Oral & Maxillofacial Surgery, Aichi-Gakuin Univ, Nagoya, Japan.

Nonsyndromic cleft lip with or without cleft palate (CL/P), cleft palate only (CPO) are common congenital anomalies. The incidence of these anomalies at birth vary from 1 in 1000 Caucasians , 2.1 in 1000 Asians, 0.3 in 1000 African races. Many studies suggest that both environmental and genetic factors are involved in the cause of CL/P and CPO. Candidate gene approaches are proving increasingly successful in identifying genes and mutations involved in nonsyndromic forms of CL/P and CPO. Linkage, association, and sequencing studies have all suggested the involvement of one or more candidate genes in this complex disorder. Recently, it is reported that several candidate genes of CL/P and CPO have been detected by using molecular biology technique. We will summarize our candidate study of MSX1, TGFB3, TGFA using 50 Japanese families. These study support a role for MSX1 in clefting and extend the analysis to Asian populations.

Power and Type I Error of Haplotype and Multiple Genotype Analysis: A Comparison of Methods. *K.K. Nicodemus, M.D. Fallin.* Department of Epidemiology, Johns Hopkins SPH, Baltimore, MD.

Several methods have been proposed to handle multiple SNP-disease association studies in family and case-control data. However, it is unknown how the power and type I error compare across methods; therefore, applied researchers must arbitrarily choose a method. Further, SNP-based haplotype or multiple-genotype analyses often involve many categories, resulting in a large number of parameters to estimate and loss of precision and power, if no data reduction strategy is taken. A common method to reduce the number of categories is to collapse haplotype/multiple-genotype categories with low frequencies into one set or to focus on one or a limited set of target haplotypes/set of genotypes, while collapsing all others into a single comparison set. Recently, methods using cladistic analysis as the basis for haplotype data reduction have been proposed for both case-control and case-parent data. The purpose of the present study was to evaluate several SNP and haplotype analysis and data reduction methods across many different scenarios, in order to compare the power and type I error rates. Data were simulated for a candidate gene study of 500 SNPs in with an average haplotype blocking structure of 1.5 blocks per gene (range 2-4 blocks/gene). For the case-parent trio design, the programs/methods evaluated included: HBAT, Multiple Genotype PDT, TRANSMIT, and ET-TDT. For unrelated case-control data, the methods evaluated included HAPLO.STAT, THESIAS, CLADHC, and COCAPHASE. For methods not using cladistic analysis, different data reduction strategies were tested, including no data reduction, collapsing categories with frequencies less than 1% and 5%, and collapsing all categories versus a single "at risk" category. Type 1 error was calculated under the null simulation, and power was calculated using statistical significance determined via permutation, as well as significance determined via the approach suggested for each method and/or implemented in its relevant software.

Identification and characterization of sequence variation at the human frizzled-1 (FZD1) locus. *J.I. Oakley¹, S.P. Moffett¹, C.S. Nestlerode¹, V.W. Wheeler², A.L. Patrick², C.H. Bunker¹, J.M. Zmuda¹.* 1) Dept Epidemiology, GSPH, Univ Pittsburgh, Pittsburgh, PA; 2) Tobago Health Studies Office, Scarborough, Tobago.

Members of the frizzled gene family encode 7- transmembrane domain proteins that are receptors for Wnts, secreted growth factors involved in embryonic development as well as several age-related diseases including cancer and osteoporosis. Very little is currently known about Wnt/Frizzled sequence diversity thus hampering genetic analyses of this pathway. To address this, we sequenced 3.3 kb of the FZD1 gene on human chromosome 7q21 including 1 kb upstream of the transcriptional start site, the 5' UTR, and the entire coding sequence using DNA samples from 48 individuals of West African descent. We found 23 SNPs and a proline repeat polymorphism using the Sequencher analysis package. 15 of the 23 SNPs in our analysis (65%) were not present in dbSNP database (build 120) whereas 6 of the 14 SNPs reported in dbSNP (42%) were not detected. We found 12 SNPs in the putative promoter region all within ~200 bp of each other, 3 SNPs in the 5'UTR, 5 synonymous SNPs, 3 nonsynonymous SNPs and a proline repeat in the coding sequence. The 3 nonsynonymous SNPs (Ala64Thr, Ala390Thr and Val497Gly) were each observed in only one individual in the heterozygous state (MAF~1%) and these were not previously described. We calculated allele frequencies and noted 7 SNPs with MAF 3%. For these SNPs, we calculated pairwise linkage disequilibrium (D) and then constructed haplotypes from the 7 SNPs using the expectation-maximization algorithm. Three major haplotypes were predicted to account for 85% of all possible haplotypes. These common haplotypes could be captured by a minimal subset of 2 tagging SNPs, rs2232157 and rs2232158. In summary, we have developed a high-resolution SNP map of the FZD1 gene region on chromosome 7q21. These analyses should facilitate future association studies and fine mapping of causal sequence variation for a range of conditions associated with disordered Wnt signaling.

A comparison of algorithms for haplotype inference: imputing missing data and reconstructing haplotypes. *P. Scheet, M. Stephens.* Statistics, Univ. of Washington, Seattle, WA.

Numerous algorithms have been proposed for inferring haplotypes from (phase-ambiguous) multilocus genotype data on unrelated individuals; however, there exist few experimentally-determined phase-known genotypic datasets upon which these methods can be applied and evaluated. The use of simulated data for this purpose may fail to accurately reflect characteristics of real populations. Here we compare the accuracy of several available algorithms applied to two tasks: imputation of missing data from real populations and inference of haplotypes from "pseudo-individuals". We obtained 50 genes that have been completely resequenced in 24 African-Americans and 23 individuals of European descent and masked 5% of the genotypes to assess the missing data imputation accuracy of the algorithms. We believe the imputation results have implications regarding the accuracy of haplotype inference, the extent of which will depend on the similarity of each task's reliance upon an underlying model for the features of population genetic data. It is unclear whether genotype data from two populations should be analyzed separately or pooled together prior to analysis; our results indicate the latter for these data, but we suggest a simple procedure by which to assess such strategies. We also apply the haplotype-inference methods to randomly-paired molecularly-determined male X chromosome haplotypes. For imputing missing genotypes, a new method which takes into account the decay of LD with distance and the order of genotyped markers outperforms the best available methods; its accuracy in inferring the X chromosome haplotypes is only slightly better.

Evaluation of simple methods to test for associations between traits and unphased haplotypes with correlated data. *S.L. Slager, S.K. McDonnell.* Mayo Clinic College of Medicine, Rochester, MN.

Schaid et al. (2002) developed a score test to test for associations between haplotypes and a trait for unrelated individuals. When haplotypes are ambiguous, their approach uses the expected score, which is a function of the posterior probabilities of each haplotype pair computed from the expectation-maximization (EM) algorithm. We investigate analytical approaches for when the data contain related individuals. This design arises in our study of prostate cancer in which our cases are from multiple-case families and our controls are unrelated and ascertained from Olmsted County, Minnesota. Using only one case from each multiplex family would be an inefficient use of resources. However, when using related individuals, two issues are of concern: computing expected scores for ambiguous haplotypes and computing the appropriate variance estimates. To circumvent these issues, we evaluate the performance of a bootstrap approach using simulations relative to an approach that ignores the familial relationship. We simulated seventeen multi-locus genotypes, using the program makesamples (Hudson, 2002), and phenotypes on each of our 160 prostate cancer pedigree structures. One thousand bootstrap samples were taken. Our initial findings demonstrate that correct type one error rates are obtained. More detailed evaluation of our approach will be presented.

Haplotype inference using a Hidden Markov Model with efficient Markov Chain sampling. *S. Sun¹, C.T.M. Greenwood², R.M. Neal¹*. 1) Department of Statistics, University of Toronto, Toronto, Ontario, Canada; 2) Program in Genetics and Genomic Biology, Hospital for Sick Children, Toronto, Ontario, Canada.

Knowledge of haplotypes is useful for understanding block structure and disease risk associations. Exact determination of haplotypes in the absence of family data is presently impractical. Hence, several methods have been previously developed for reconstructing haplotypes from population data. We have developed a new population-based method using a Hidden Markov Model (HMM) for the source of the ancestral haplotype segments. Parameters of the model are inferred by Bayesian methods using Markov Chain Monte Carlo (MCMC). Crucial to the efficiency of the Markov Chain sampling is the use of an efficient forward-backward algorithm for sampling state sequences of the HMM. We have used the model to reconstruct the haplotypes at 103 loci of 129 children in the data set of Daly et al (Nature Genetics 2001, 29:229-232), for which family-based reconstruction is available. Our haplotype reconstruction method does not require division into small blocks of loci and produces results that are quite close to the ones from the family-based reconstruction.

Development of an algorithm to test, by nonparametric methods, the association between haplotypes and quantitative phenotypes using MCMC algorithm. *R. Takemura*¹, *N. Kamatani*². 1) Japan Biological Information Research Center, Japan Biological Information Consortium, Tokyo, Japan; 2) Division of Genomic Medicine, Department of Applied Biomedical Engineering and Science and Institute of Rheumatology, Tokyo, Japan.

We developed an algorithm to test the association between haplotypes and quantitative phenotypes using EM-algorithm. This algorithm was implemented as a computer program QTLHAPLO (Shibata et al. Genetics in press). Taking the genotype and phenotype data as input data, QTLHAPLO estimates the parameters (such as population means) of the distributions for the two groups of the subjects classified according to a haplotype. At the same time, QTLHAPLO tests the hypothesis that the parameters (and the distributions) are different between the two groups. Dominant, recessive and additive modes can be assumed in the program. In that algorithm, the phenotype is assumed to follow a certain distribution such as normal distribution. However, in many cases, the true distribution of the quantitative phenotype is not clear. In the present study, we propose a nonparametric method to overcome the problem in QTLHAPLO. In our new algorithm, Markov Chain Monte Carlo MCMC algorithm is used. First, population haplotype frequencies are sampled using the numbers of haplotypes in the sample based on the Dirichlet distribution. Next, the diplotype configuration of each subject is stochastically determined based on both the population haplotype frequencies and the genotypes. The subjects are divided into two groups according to the haplotypes possessed by the subjects, and it is tested whether the medians of the quantitative phenotypes of the two groups are different using the nonparametric method. We have adopted the two-sample test by Kolmogorov-Smirnov and Mann-Whitney's U test. After generating sufficient numbers of MCMC samples, the nonparametric tests were performed using the samples. The overall test was performed using the average of the values of the statistic. By the simulation, we have shown that the present method can be applied to the quantitative phenotypes that are not expected to follow normal distributions.

Linkage disequilibrium analysis of microsatellite markers and HLA class I alleles associated with Seronegative Spondyloarthropathies in South India. *R. Thomas, M. Banerjee.* Human Molecular Genetics Laboratory, Rajiv Gandhi Centre for Biotechnology, Trivandrum, India.

Seronegative Spondyloarthropathies (SpA) constitute a group of disorders with common genetic and clinical characteristics. A genetic predisposition associated with HLA B*27 is common to this set of complex disorders. The strength of the disease association with B*27 varies markedly among the various spondyloarthropathies in different ethnic communities. HLA haplotype analysis coupled with microsatellite mapping in the HLA class I region could identify regions of disease associations and facilitate better diagnosis of the different spondyloarthropathies. In the present study we have used association studies to ascertain the association of microsatellite markers with various HLA class I alleles in SpA patients. We have used strict clinical, radiological and serological criteria to select 69 patients with SpA. We compared these patients with 78 healthy normal individuals from the same ethnic background. HLA class I alleles were identified using the DNA based PCR-SSP method while five microsatellite markers including C1_2_5, C1_4_1, C1_2_A, MIB and STR MICA were analysed using Genescan.

The HLA alleles present in the control samples with highest frequency were B*07 (0.192), Cw*07 (0.25), A*02 (0.186) while B*27 (0.232), Cw*14 (0.203), A*24 (0.217) had the highest frequency in the SpA patient group. The bi-locus haplotype B*27-Cw*02 occurred with significantly high frequency in the SpA samples. The microsatellite alleles occurring with a high frequency in the control samples were C1_2_5 (196 bp), C1_4_1 (217 bp), C1_2_A (232 bp), MIB (336 bp) and STR MICA (A5.1) while in the SpA samples it was C1_2_5 (178 bp), C1_4_1 (213 bp), C1_2_A (232 bp), MIB (340 bp) and STR MICA (A4). We also identified a significant association of an extended six-point haplotype block B*27 - Cw*02 - STR MICA (A4) - C1_2_5 (178 bp) - C1_4_1 (213 bp) - MIB (340 bp) in the SpA samples.

Accuracy of haplotype frequency estimates in samples of families and unrelated individuals. *Z. Ye, J. Wigginton, G. Abecasis.* Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor, MI.

Haplotype frequency estimates are important for genetic association and linkage disequilibrium studies. These estimates can be derived from family samples or samples of unrelated individuals. Here we compare the relative accuracy of haplotype frequency estimates resulting from different sampling schemes. We used coalescent simulations to generate samples of haplotypes, and varied the recombination rate, proportion of missing data, as well as the number and type of families collected for genotyping. We used a generalization of the expectation maximization algorithm for estimation of haplotype frequencies that can handle simple family structures, with or without missing data. In all settings, we find that missing data and historical recombination reduce the accuracy of haplotype frequency estimates. In addition, we find a small bias on estimates of haplotype frequencies. Typically, estimates show a downward bias for rare haplotypes and an upward bias for more common haplotypes. For a fixed genotyping effort, it appears that samples of trios provide more accurate haplotype frequency estimates than samples of unrelated individuals, when there is historical recombination and/or substantial missing data, while samples of unrelated individuals have a slight advantage under circumstance without recombination and with no missing data. These results will be important for investigators planning to design or carry out gene-mapping and linkage disequilibrium studies in human populations.

Analysis of multiple error rates in haplotype-based association studies. *L. Beckmann*¹, *C. Fischer*², *J. Chang-Claude*¹. 1) Dept Clinical Epidemiology, German Cancer Research Center, Heidelberg, Germany; 2) Dept of Human Genetics, Univ. of Heidelberg, Germany.

Large-scale association studies hold substantial promise for gene mapping in complex disease. These studies involve analysis of multiple genetic markers whereby various hypotheses are tested simultaneously, resulting in both inflated false positives and failures to detect true associations. Multiple error correction is thus necessary and is important when comparing power of different methods. We aim to contrast different strategies for controlling multiple error levels in case-control scenario. We applied three strategies: 1. A permutation procedure, where multiple error is controlled either by the *step-down maxT* or the *step-down minP* adjusted p-values (Westfall and Young, Resampling-based multiple testing. John Wiley & Sons 1993). 2. The rank truncated product of p-values (*RTP*) by Dudbridge and Koeleman (Genetic Epidemiol 25:360-6, 2003). 3. The method proposed by Hoh et al. (Genome Res 11:2115-9, 2001). We performed simulation studies under the null hypothesis for different models, varying the number of genetic markers, allele frequencies and level of linkage disequilibrium. Three statistical methods for gene mapping were analyzed: 1. A new method for gene mapping based on Mantel statistics to correlate phenotypic similarity with genetic similarity, measured as the shared length between two haplotypes at a putative disease locus. 2. The single-point χ^2 -test. 3. The χ^2 -test for overlapping sliding windows consisting of 3-marker haplotypes. For a fixed pointwise error level of 5% at each marker position, the Mantel statistic showed the lowest multiple error. Multiple error was more inflated for the haplotype-based χ^2 -test and was even stronger for the single-point χ^2 -test. Permutation procedures are straightforward if the distributions of the test statistics are analytically known. If empirical p-values have to be estimated by permutation, the *step down minP* procedure must be used. *RTP* has the disadvantage that a parameter has to be chosen in advance. Our results can serve as a guideline for multiple error correction in the analysis of large-scale association studies.

Association of vitamin-D receptor (VDR) gene polymorphism (Fok-I) with calcium oxalate nephrolithiasis. *H.K. Bid¹, A. Kumar², R. Kapoor¹, R.D. Mittal¹*. 1) Department of Urology, SGPGIMS, Lucknow, Uttar Pradesh, India; 2) Department of Biochemistry, GSVM, Medical Kanpur, Uttar Pradesh, India.

Urolithiasis is a multifactorial and polygenic disease. The etiology of idiopathic calcium oxalate nephrolithiasis is heterogeneous, involving both genetic and environmental factors. The formation of urinary stones is reported to be associated with the genetic polymorphism of vitamin-D receptor (VDR) gene. In order to evaluate the role of VDR gene start codon Fok-I polymorphism in stone formation, we aimed to explore the association between calcium stone disease and the Fok-I polymorphism in North Indian population. A control group comprising of 166 healthy individuals (age range 22-58 years) and 138 patients with calcium oxalate stone disease (age range 21-72 years) were included in the study. The polymorphism was detected by using PCR based restriction analysis. An unexcisable band of 265 bp (CC) and two bands of 169 bp and 96 bp (TT) were obtained by Fok-I restriction digestion. The frequency of the genotype CT was observed to be higher in the patients (76.8%) as compared to the healthy controls (50.6%). However, the frequency of the genotypes CC and TT were higher among healthy controls (46.4% and 3%) compared to patients (21.7% and 1.5%). The results suggest a statistically significant association between calcium oxalate stone disease and VDR gene Fok-I polymorphism. It is also suggestive of a candidate gene, which could be used as a genetic marker contributing to risk factor in calcium oxalate stone disease.

Convergence of genome-wide expression analysis and genome-wide linkage analysis identifies candidate genes for atherosclerosis. *E.R. Hauser, S.G. Gregory, D. Seo, A. Dobra, E. Iversen, R. Karra, C.S. Haynes, J. Stenger, H. Xu, L. Wang, L. Huang, M. West, M. Sketch, J.M. Vance, W.E. Kraus, P.J. Goldschmidt, for the GENECARD and AGENDA Investigators.* Duke University, Durham, NC.

Genome-wide expression analysis of coronary tissues and genome-wide linkage analysis in families with early-onset CAD are unbiased methods to identify candidate genes for CAD. Both methods can result in a large number of candidate genes. Identifying genes that appear on both lists may reveal the strongest candidates. Sixty-eight genes were identified as strongly predictive of atherosclerosis from expression analysis using the Affymetrix U95 chip on 77 aortas. Regions of linkage were identified from results for 420 families in the GENECARD study. Five SNPs per gene were selected for genotyping, which has been completed on 63% of the SNPs. These genes were tested for association in an independent set from the CATHGEN sample of the Duke Catheterization Lab Database. Subjects were selected according to their CAD index (CADi), a validated angiographical measure of the extent of coronary atherosclerosis: 301 young affected subjects (age < 55, CADi > 32), 168 older affected subjects (age > 55, CADi > 74) and 204 unaffected elderly subjects (age > 60, CADi < 23). To screen for association p-values were calculated by logistic regression adjusting for sex and ethnicity for each SNP. Among 43 expression candidates with at least 1 SNP genotyped, 37 were not in regions of linkage and 6 were in regions of linkage. Of the 6 genes in regions of linkage, 5 (83%) showed evidence for association ($p < .05$) with at least one SNP. The 5 genes (chromosome, expression rank, pvalue) are: LAM5 (1, 38, .04); GM2A (5, 14, .02); BPAG (6, 29, .03); GPNMB (7, 15, .02); MMP9 (20, 44, .04). Haplotype analysis shows that approximately of these genes show evidence for haplotype associations. Of the 37 genes not in regions of linkage 13 showed evidence of association ($p < .05$) with at least one SNP (35%). The convergence of evidence from multiple genome-wide sources is a useful tool for identifying the most important candidate genes for additional analyses.

Affected males transmit multiple sclerosis more often to children than affected females: The Carter effect. *O.H. Kantarci¹, L.F. Barcellos², J.R. Oksenberg³, E.J. Atkinson¹, R.R. Lincoln³, M. deAndrade¹, B.G. Weinshenker¹, S.L. Hauser³.* 1) Neurology, Mayo Clinic, Rochester, MN; 2) Neurology, University of California, Berkley, CA; 3) Neurology, University of California, San Fransisco, CA.

Women to men ratio is 2:1 in multiple sclerosis (MS)(Mayr et al. Neurology 2003; 61(10): 1373-77). If this difference was largely genetic rather than environmental, men need a larger load of susceptibility genes to overcome this relative resistance compared to women and would transmit the disease more often to their children, a phenomenon known as the Carter effect (Carter. Br Med Bull 1961; 17:251-54). We studied the presence of Carter effect in MS. We identified 105 affected individuals with an affected father or mother (38 affected index parents) from 482 affected individuals in 185 pedigrees that were previously collected (Haines et al. Nature Genetics 1996; 13(4): 469-71). We compared transmission of MS from affected men with transmission from affected women. Significance was assessed with Fisher's Exact test. The ratio of women to men were: of all affected individuals 2.4 (342:140); of all unaffected individuals 1.0 (603:612); of index parents 2.2 (26:12), of their affected offspring 2.9 (29:10), of their unaffected offspring 1.8 (42:24); of unaffected parents 0.95 (291:305), of their affected offspring 2.5(651:260) and of their unaffected offspring 1.04 (702:672). This distribution shows that the increased frequency of MS in women is independent of parents' affected status and the threshold to develop MS in women is lower than men. Men with MS transmitted the disease to their children more often (transmitted: 18, not transmitted: 18) than affected women (transmitted: 21, not transmitted: 48) ($p=0.049$;OR: 2.29, 95%CI: 1.00-5.24). To eliminate confounding by gender of the offspring, the analysis was repeated considering only transmission to daughters. Affected men transmitted MS more often to their daughters (transmitted: 14, not transmitted: 10) than affected women (transmitted: 15, not transmitted: 32)($p=0.032$;OR: 2.99, 95%CI: 1.08-8.26). Our results confirm the presence of Carter effect in MS and suggest that larger genetic loading in men may result in excess paternal transmission.

A Pattern Discovery-Based Method for Detecting Multi-Locus Genetic Association. Z. Li¹, A. Floratos¹, D. Wang¹, A. Califano^{1,2}. 1) Computational Genetics, First Genetic Trust, Inc, Rutherford, NJ; 2) Department of Biomedical Informatics, Columbia University, New York, NY.

Methods to effectively detect multi-locus genetic association are becoming increasingly relevant in the genetic dissection of complex trait in humans. Current approaches typically consider a limited number of hypotheses, most of which are related to the effect of a single locus or of a relatively small number of neighboring loci on a chromosomal region. We have developed a method that is specifically designed to detect genetic association involving multiple disease-susceptibility loci, possibly on different chromosomes. Our approach relies on the efficient and exhaustive discovery of patterns comprising spatially unrestricted polymorphic markers and on the use of appropriate test statistics to evaluate pattern-trait association under case-control design.

A genotypic pattern describes a genetic signature (a panel of markers with particular genotypes) that is shared by numbers of individuals in the case and control populations. The statistically significant enrichment of a genotypic pattern in case population vs. control population could suggest trait association. One advantage that the pattern discovery-based method might have over existing single marker-based or haplotype-based methods is that trait-associated genetic signatures involving multiple non-continuous markers can now be identified. Power calculations using two multi-locus disease models, two sample sizes, and five affected genotype frequencies demonstrate significant gain of power by using this method in detecting multi-locus genetic association when compared to a standard single marker analysis method. When analyzing a Schizophrenia dataset, we confirmed a previously identified gene-gene interaction (DAO-G72 interaction). In addition, a less conspicuous association involving different markers on the same DAO and G72 genes was also identified, implicating genetic heterogeneity.

The 3020insC Mutation of the CARD15/NOD2 Gene Predicts Risk of Early Surgery in Pediatric Crohns Disease.

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Large-scale linkage and association studies have been frequently employed to uncover the genetic underpinnings of complex diseases. Ultimately, the question remains whether these methods identify genes with significant clinical or prognostic relevance. We recently demonstrated an association of the 3020insC polymorphism of the CARD15/NOD2 gene to pediatric-onset Crohns Disease (CD) in a prospective cohort of Caucasian children. As a subset of children with CD progress to severe disease requiring surgical intervention, our goal was to determine if the 3020insC SNP could identify a subset of children at high risk for surgery who would benefit from early genetic testing and more aggressive therapy. In a prospective study, 163 Caucasian children with CD were recruited with parents and followed for up to 70 months. We used a Cox Proportional Hazards model, stratified according to the presence or absence of the 3020insC allele, to determine the effect of this mutation on surgery-free survival. A test of the proportionality assumption was performed using the Schoenfeld residuals. The Cox PH model demonstrated an unadjusted hazard ratio of 6.07 (95% C.I. 2.76-13.37, $p < 0.0001$), indicating that the hazard for surgery is six-times greater in children with the 3020insC SNP than in those without it. The hazard ratio changed marginally when adjusted for disease location and presentation at diagnosis (5.83, 95% C.I. 2.62-12.98, $p < 0.0001$). This prospective study demonstrates that children positive for the 3020insC variant are at a significantly higher risk for early surgery than children without the mutation, suggesting that this variant could act as a prognostic factor for early surgery in Caucasian CD children. While this study is specific to the particular case of pediatric-onset CD, we believe it might also serve as an example of how gene identification strategies can lead to new clinically important prognostic markers.

Correcting for population stratification using genomic control. *D. Shmulewitz*^{1, 2}, *J. Zhang*², *D.A. Greenberg*^{1, 2, 3}.
1) Department of Psychiatry; 2) Division of Statistical Genetics, Columbia University, New York, NY; 3) New York State Psychiatric Institute, New York, NY.

Case-control association studies in mixed populations can result in spurious disease-marker associations (i.e. increased false positive rate, FPR) if both disease prevalence and marker frequencies differ in the subpopulations. The Genomic Control (GC) approach uses neutral loci to correct the χ^2 to reflect the nominal p-value of 5%. Previously, in simulated association studies, we showed that applying GC using sets of markers with low average allele frequency differences (M) between the component subpopulations can restore the expected 5% FPR. Here we study the effect on GC when a few of the markers have high allele frequency differences (outlier markers). We simulated two populations with different disease and marker (1000 SNPs) frequencies and without any disease-marker association. We generated mixed populations with disease ratios (DR, disease prevalence in population1/prevalence population2) of 0-1. We selected sets of 100 markers with a small number of outlier markers. We drew 100 datasets of 100 cases/controls and calculated the χ^2 -statistic for disease association with each marker. We applied two genomic control procedures, dividing by the mean χ^2 or median- $\chi^2/0.456$, to see how effectively these procedures eliminated excess false positives. When outlier markers were present, correcting by the mean resulted in corrected FPRs (cFPRs) close to 5%. However, when the DRs were smaller (0.2), the cFPRs were conservative (4%). In contrast, correcting by the median/0.456 resulted in cFPRs that were quite anticonservative (6%) for most DRs. The conservativeness of the mean correction and the anti-conservativeness of the median correction both became more pronounced as the number of outlier markers increased. M also affected the median correction. GC, particularly using the mean correction, might be useful in certain situations to correct population stratification. In other situations, GC may lead to a notable loss of power to detect a true association (conservative) or may not completely eliminate the spurious associations (anticonservative).

Cladistic Grouping of Haplotypes in Association Analysis. *J.Y. Tzeng*^{1,2}. 1) Department of Statistics, NC State University, Raleigh, NC; 2) Bioinformatics Research Center, NC State University, Raleigh, NC.

Haplotypes represent underlying polymorphisms more than single SNPs, and are considered as a more informative format of data in studying genetic association with phenotypes. To model haplotypes, it requires high degrees of freedom, which could decrease power and limit the model capacity to incorporate other complex effects such as interactions. Even within haplotype blocks, high degrees of freedom can still be a concern unless one chooses to discard rare haplotypes. To increase the efficiency and power of haplotype analysis, we adapt the concepts of cladistic analysis and propose a grouping algorithm to cluster rare haplotypes to their corresponding ancestral haplotypes. The algorithm determines the cluster basis by reserving common haplotypes using a criterion built on Shannon information content. Each haplotype is then assigned to its appropriate clusters probabilistically according to the cladistic relationship. Through this algorithm, we calculate the maximum likelihood estimates of the regrouped haplotype frequencies from genotypes, and perform association analysis based on groups of haplotypes. Simulation results indicate increase in power for tests based on haplotype clusters obtained by the proposed algorithm comparing to tests based on original haplotypes.

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Native South American genetic variation and its relationship to tuberculosis prevalence: the vitamin D receptor and SLC11A1. *A.K. Wilbur¹, L. Salter¹, A.M. Hurtado¹, K.R. Hill¹, A.C. Stone².* 1) Univ New Mexico, Albuquerque, NM; 2) Arizona State University, Tempe AZ.

Tuberculosis incidence among Native American groups since European contact has been especially high, and our ethnographic and molecular data indicate prevalence greater than 50% among the Ach and Av of Paraguay. Despite extensive evidence that host genetics help determine susceptibility and resistance to tuberculosis in various populations around the world, little research has been conducted on Native American susceptibility. This project examines the potential interplay of host genetics and environmental factors in tuberculosis susceptibility in Ach and Av. Using molecular biological techniques, sequence data and SNPs at two candidate loci (the vitamin D receptor and SLC11A1, also known as NRAMP1) are examined for potential association with tuberculosis. Our extensive longitudinal, demographic data for Ach allow for co-analysis of factors such as sex, age, and proximity to non-Native communities. Here we report on our preliminary findings.

Genetic models of tumor necrosis factor- expression and tuberculosis susceptibility. *C.M. Stein^{1,2,3}, R.D. Mugerwa^{2,4}, W.H. Boom², K.D. Russo¹, R.C. Elston¹, C.C. Whalen^{1,2,3}, S.K. Iyengar¹.* 1) Epidemiology & Biostatistics, Case Western Reserve Univ, Cleveland, OH; 2) Tuberculosis Research Unit, CWRU, Cleveland, OH; 3) Epidemiology of Infectious Diseases Center, CWRU, Cleveland, OH; 4) Makerere University School of Medicine, Kampala, Uganda.

Tuberculosis (TB) is a major public health problem in sub-Saharan Africa. Several studies suggest there is a host genetic component for TB risk. TB is a complex multifactorial genetic trait because of its heterogeneity in disease presentation, variation in severity and length of latent infection, long-term disease risk, and dependence on exposure to *M. tuberculosis*. We examined levels of tumor necrosis factor- (TNF) as an intermediate immunologic phenotype for TB because it is a key cytokine in TB pathogenesis, is correlated with the natural history of TB, and has previously demonstrated high heritability (68%). We conducted a household contact study of 172 families comprising 831 people in Kampala, Uganda, a TB-endemic community. We used path analysis to partition the components of variance due to genetic and shared environmental effects. Results suggested that TNF expression is influenced by genes (34-66% heritability), is not strongly influenced by shared environment, and is possibly influenced by gene-environment interaction. Segregation analyses showed that TNF follows a major gene model with three underlying means, in which the heterozygote mean was less than either homozygote mean, consistent with heterozygote advantage, and heterogeneity of transmission across generations was accounted for by age and HIV serostatus. Candidate region linkage analyses suggest a minor role of MHC loci in TNF regulation and TB susceptibility (D6S439, $p=.04$). Our new path model increases power to detect linkage and illustrates previously unexplored components of genetic risk to TB. Analysis of underlying immunological components of TB will yield insights into TB genetics by determining deficiencies in host immunity that ultimately predispose individuals to develop TB. Planned linkage analysis of additional candidate genes will enable the identification of early immune responses that lead to host susceptibility.

Segregation analysis of Restless Legs Syndrome (RLS). *R.A. Mathias¹, W.A. Hening², M. Washburn³, R.P. Allen³, S. Lesage³, A.F. Wilson¹, C.J. Earley³.* 1) Inherited Disease Research Branch, NHGRI/NIH, Baltimore; 2) UMDNJ-RW Johnson Medical School, New Brunswick; 3) Johns Hopkins University, Baltimore.

RLS is a sensorimotor disorder that has a prevalence of ~7% in the US. A diagnosis of RLS requires establishing an urge to move localized to the legs which is provoked by rest, relieved by activity, and accentuated late in the normal wake period and early sleep period. A segregation analysis done in German families with probands having an age of onset below 30 supported a dominant pattern of inheritance [Winkelmann J et al., *Ann Neurol*, 2002, 52:297-302]. In this study, probands were selected from consecutive RLS patients from the Neurology and Sleep clinics of the Johns-Hopkins Bayview Medical Center. Patients willing to have first and second degree relatives contacted were included. An RLS diagnosis was made in those who had the four diagnostic features of RLS [Allen RP et al., *Sleep Med*, 2003, 4:101-119] and whose symptoms could not be explained by an alternate diagnosis. There are 2060 subjects in 76 pedigrees with 534 sibships. The average pedigree size is 27 (range 6-90) with an average of 4.8 generations. Phenotype data is available on 590 subjects of whom 281 have a confirmed diagnosis of RLS. Initial examination of simple segregation ratios suggests an autosomal dominant mode of inheritance of RLS that appears to be more pronounced in families with an earlier proband age of onset. A formal segregation analysis of these pedigrees is ongoing.

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GARFIELD: A Graphical Analysis and Reorganizing Interface for Linkage Disequilibrium. *J. Cazier, D.F. Gudbjartsson, M. Stefánsson, A. Kong.* Dept Statistics, deCode Genetics, Reykjavik, Iceland.

Linkage and association studies require the understanding of the underlying structure of the chromosome, an important part of which is captured by the linkage disequilibrium (*LD*) patterns. A preamble to any research over a specific region has to be its in-depth analysis, and therefore efficient tools to address the question are necessary. Coupling the genotype data from the investigator to the releases from the **Hapmap** project provides an excellent platform to identify the *LD* structure of any region. We have developed **GARFIELD**, a user-friendly, powerful, yet modular, graphical interface to make maximum use of *LD* patterns in data.

GARFIELD works with either genotypes or direct *LD* information of pre-processed data, available in text file. Typically pairwise D' , R^2 and p -val output from the **Nemo** program by **deCODE Genetics** are used. This software can quickly handle small and large data sets with various level of zooming. *LD* blocks can be easily identified and further analysed to determine haplotype diversity whenever genotypes are the input. **GARFIELD** is not only an interface to plot and analyze *LD*, but it also contains all the options to prepare ready-to-publish images.

A feature of the program is the re-organizer which allows the identification and correction of map misplacements and genotyping errors. This is possible by allowing manual permutations of the marker position in the map. Moreover an algorithm provides permutations to optimize the block structure for scores based on quantities like D' , R^2 , p -val, index- and physical- distance between marker pairs.

All sources and executables are freely available upon request.

Genetics and Homocysteine in Obese West Virginians. *M. Flood*², *J.M. Chappell*³, *C. Clark*², *S. Hesson*¹, *B. Hill*⁴, *J. Jeong*¹, *J. Nash*¹, *M. Ramey*¹, *J. Roth*¹, *P. Sekar*⁶, *B. Kahle*⁶, *G. Wright*⁵, *T. Green*⁵, *M. Studney*⁵, *P. Wehner*⁵, *E.E. Murray*¹. 1) IST, Marshall University, Huntington, WV; 2) Biology, Fairmont College, Fairmont, WV; 3) CLS, MU, Huntington, WV; 4) Biology, West Virginia State University, Institute, WV; 5) Joan C. Edwards School of Medicine, MU, Huntington, WV; 6) Biology, MU, Huntington WV.

We genotyped two functional MTHFR variants (MTHFR 677; MTHFR 1298) and Factor V Leiden (FFL 1691) in 581 subjects. All loci are on chromosome 1. The MTHFR variants (677T and 1298C) modestly elevate plasma homocysteine (HSC) with consumption of a low folate diet. HSC level is a significant risk factor for patients with CVD. FFL 1691A causes resistance to activated protein C, increasing venous thrombosis risk. Although obesity increases CVD risk, the role genotype plays in elevated HSC in the obese is unknown. 77.2% of subjects are obese (BMI 30) and 10.9% are overweight (BMI 25-30). There is no significant difference between genotype distributions in the obese compared with a WV population of European origin not selected for obesity.

MTHFR 677	MTHFR 1298	FFL 1691
CC 55.2%	AA 47.5%	GG 94.5%
CT 38%	AC 43.5%	GA 5.5%
TT 6.8%	CC 9%	AA 0%

*MTHFR 1298A allele is linked to MTHFR 677T, and 1298C allele with 677C (p 0.001). LD is observed between MTHFR 1298A and FFL 1691G and between 1298C and 1691A (p 0.05). FFL 1691 and MTHFR 677 loci had no LD. Only MTHFR1298 genotype positively correlates with obesity. 1298AA genotype is higher in normal individuals and 1298CC genotype is lower in the obese (p 0.036), indicating a possible survival benefit for 1298 AA genotype.

Assessment of Power for Association Studies to Finely Map a Locus Identified by Linkage Analysis. *R. Hanson, W. Knowler.* DAES, NIDDK, Phoenix, AZ.

Association (e.g. case-control) studies are often used to finely map loci identified by linkage analysis. We investigated the influence of various parameters on power and sample size requirements for such a study. Calculations were performed for various values of a high-risk functional allele (f_A), frequency of a marker allele associated with the high risk allele (f_1), degree of linkage disequilibrium between functional and marker alleles (D') and trait heritability attributable to the functional locus (h^2). The calculations show that if cases and controls are selected from equal but opposite extreme quantiles of a quantitative trait, the primary determinants of power are h^2 and the specific quantiles selected. For a dichotomous trait power also depends on population prevalence (P_D). For example, for $h^2=0.05$, $P_D=0.1$ and $D'=1.0$, the sample size required to detect an association with the marker at $p<0.01$ is 266 if $f_A=f_1=0.1$ (assuming an equal number of cases and controls) and 416 if $f_A=f_1=0.9$. Power is optimal if functional alleles are studied ($f_A=f_1$ and $D'=1.0$) and can decrease substantially as D' diverges from 1.0 or as f_1 diverges from f_A (even with D' near 1.0). These analyses suggest that association studies to finely map loci will most likely be successful if potential functional polymorphisms can be identified *a priori* or if markers are typed to maximize available haplotypic diversity. In the absence of such information, expected minimum power at a given location for a given sample size can be calculated by specifying a range of potential frequencies for f_A (e.g. 0.1-0.9) and determining power for all markers within the region with specification of the expected D' between the markers and the functional locus. As an illustration of this method, calculations were performed for a body mass index mapping project consisting of 662 single nucleotide polymorphisms in 24 Mb. Regions differed by marker density and allele frequencies. Thus, in some, power was near its theoretical maximum and little additional information is expected from additional markers, while in others, additional markers appear to be necessary. These methods may be useful for assessing the progress of fine-mapping studies.

Efficiency and Robustness of a Family-Based Conditional Likelihood Ratio Test for Association. *J.P. Lewinger¹, S.B. Bull^{1, 2}*. 1) Samuel Lunenfeld Research Institute, Toronto, ON, Canada; 2) Dept. of Public Health Sciences, Univ of Toronto, Toronto, ON, Canada.

In a previous report (Lewinger and Bull, 2003 *Am J Hum Genet* 73 (5S); 613) we introduced a likelihood ratio test of linkage in the presence of allelic association, based on the conditional framework of Rabinowitz and Laird (D. Rabinowitz, N. Laird, *Hum Hered* 2000). This test efficiently uses all available nuclear family information, including genotypes from both affected and unaffected siblings, as well as from homozygous parents. The test statistic is a conditional likelihood ratio based on a standard two point linkage model that includes an allelic association parameter. Estimation of the model parameters guarantees asymptotically optimal power within the set of alternatives specified by the model. The test is immune to population stratification and can be applied to families with any pattern of missing information. With complete parental genotypes, the null distribution of the test statistic is obtained by a within-family randomization scheme in which the phenotypes of all individuals are kept fixed, while the children's genotypes are randomized according to Mendelian transmission probabilities. With missing parental genotypes, a constrained randomization of the children's genotypes is required (Rabinowitz and Laird). We determined the maximum theoretical power attainable by any conditional test against a single point alternative and found through simulation that for a wide range of scenarios the proposed test has near optimal power, outperforming the TDT when data were complete and the SDT when parental genotypes were missing. In recent simulation studies we evaluated the performance of the tests under alternatives outside the likelihood model, particularly mixed populations with differing allele frequencies. We found that although the power gains are more modest, our proposed test still outperforms the TDT and the DTD in most scenarios.

Influence of Linkage Disequilibrium of High-Density SNPs on Prostate Cancer Linkage Results. *S. McDonnell¹, S. Slager¹, J. Guenther¹, G. Christensen¹, C. Rosenow², C. Hilker¹, J. Cunningham¹, D. Schaid¹, S. Thibodeau¹.* 1) Mayo Clinic College of Medicine, Rochester, MN; 2) Affymetrix, Inc., Santa Clara, California 95051.

Most genetic linkage packages assume that the markers analyzed are in linkage equilibrium. This assumption is likely to be invalid when analyzing a large number of genetic markers, such as when analyzing single nucleotide polymorphisms (SNPs) from SNP arrays. As part of a genome-wide scan for prostate cancer using the Early Access Affymetrix Mapping 10K array, we performed linkage analyses with and without SNPs in high linkage disequilibrium (LD) to investigate the effect of LD on linkage results. We computed the pair-wise LD measures r^2 and $|D|$ between all pairs of SNPs that were within 5 cM distance from each other. A threshold was set for each measure to define a cluster of SNPs in high LD. From each cluster, the most informative SNP was selected for linkage analysis. We considered thresholds of 0.01, 0.2, 0.4, 0.6, and 0.8. Multipoint model-free LOD scores, based on the Kong and Cox exponential model, were calculated for the full set of SNPs and for subsets of SNPs after removing those in high-LD, as defined by the threshold. Linkage information content was estimated using the entropy information described by Kruglyak et al. All analyses used Merlin software. Over all chromosome positions, the average information content for the full set of SNPs was 63%. The information content was similar when moderate to high levels of LD ($r^2 > 0.2$ and $|D| > 0.6$) were excluded, but dropped to 46% when only one SNP within a 5 cM region was used ($r^2 > 0.01$). Several chromosomes exhibited lod score peaks using the full set of SNPs that either disappeared or were much less striking when markers with moderate to high levels of LD were removed. For instance on chromosome 4, a lod score of over three, observed with the full set of SNPs, was reduced to a lod score of less than one when SNPs were removed using $r^2 > 0.4$ criterion. These results suggest that including high-LD SNPs in linkage analyses can lead to inflated lod scores, however removing all evidence of LD can unnecessarily decrease the information content.

Apolipoprotein E polymorphism, plasma lipids and coronary heart disease in North West India (Punjab): An Asian perspective. *P. Singh¹, M. Singh¹, S.S. Mastana², D.P. Bhatnagar¹.* 1) Dept of Human Biology, Punjabi University, Patiala, 147002 Punjab, India; 2) Human Genetics Lab., Department of Human Sciences, Loughborough University, Loughborough, LE11 3TU, UK.

The distributions of plasma lipids, lipoproteins and genetic polymorphism of Apolipoprotein E were investigated in angiographically diagnosed coronary heart disease patients (n =78) and controls (n =50) of North West State of India (Punjab). HDL-C and LDL-C/HDL-C ratio were found to be significantly different in E3-3 and E3-4 genotype patients (F = 60.11 & 23.07, p < 0.05) and controls (F=47.94 & 9.40, p < 0.05) respectively however, non-HDL-C and LDL-C were found to be dissimilar in E3-4 genotypes of patients (F = 4.72, p < 0.05) and controls (F= 4.80, p < 0.05). ApoE3-4 subjects were at 4 times higher chance of afflicting to 3 vessel disease (OR = 4.03, 95% CI; 0.91-18.83, p < 0.05). Overall the higher frequencies of E3-4 genotype reflected it to be a modest indicator of angina (OR = 2.06, 95%CI; 0.49-9.18), myocardial infarction (OR = 2.19, 95%CI; 0.41-11.86), diabetes (OR = 1.82, 95%CI; 0.29-11.01), 1 vessel (OR = 2.03, 95%CI; 0.32-12.45) and 2 vessel disease (OR = 2.88, 95%CI, 0.58-14.62). The frequencies of ApoE*2, ApoE*3 and ApoE*4 alleles in patients and controls were found to be 0.038, 0.865, 0.097 and 0.06, 0.87, 0.07 respectively. An opposite and unforeseen trend of ApoE3-4 and ApoE3-2 genotypes was observed when subjected to geographical correlates of North latitude and East longitude. E3-2 genotype was observed to be decreasing towards North ($y = -0.0017x + 0.158$, $r = 0.3429$, $p < 0.05$) where CHD prevalence is low and reverse trend was exhibited by E3-4 ($y = 0.0009x + 0.113$, $r = 0.1288$, $p > 0.05$), however the association of percent CHD mortality with ApoE3-2 and ApoE3-4 genotypes ($r = 0.3090$, 0.3460 respectively, $p < 0.05$) was found to be significantly associated in Asia.

Program Nr: 2955 from the 2004 ASHG Annual Meeting

Statistical Power of Extreme Discordant Phenotype Design. *G. Zhang, D.W. Nebert, R. Chakraborty, L. Jin.* Dept Environmental Health, Univ Cincinnati, Cincinnati, OH.

Population based case-control study of association between disease and genotype has become a major tool in the search for genes underlying complex diseases. And it has been proposed that power to detect association can be increased by using selected cases and/or controls of extreme discordant phenotype (EDP). The advantage of EDP methodology is intuitive, therefore, a rigorous mathematical analysis on its statistical performance is necessary. We developed a statistical framework to calculate the power of EDP based on quantitative genetics model and studied the statistical performance of EDP under a range of conditions. We first studied the genotype frequency changes of a trait locus under truncation selection and presented formulas for calculating power and sample size for EDP case-control design. We showed that EDP methodology is a powerful method for genetic association study both in terms of statistical power and robustness. And better statistical performance can be achieved when cases and controls are both selected from extremes and are well balanced. As a special case, we also showed EDP design by controlling on environmental exposure will also increase the power to detect association, especially when both exposure rate and relative risk are high. And finally, by simulation, we observed EDP is less sensitive to false positive discovery derived from population stratification.

Assessment of the impact of using sex-averaged genetic maps in multipoint linkage analysis when IBD status is incompletely known. *T.E. Fingerlin*¹, *G.R. Abecasis*², *M. Boehnke*². 1) University of Colorado Health Sciences Center, Denver, CO; 2) Center for Statistical Genetics, University of Michigan, Ann Arbor, MI.

The ratio of male and female recombination rates has been shown to vary dramatically across the human genome (Broman et al. 1998, Kong et al. 2002). Despite these sex differences in the genetic map, most linkage analyses use sex-averaged genetic maps for multipoint analyses. We investigated the impact of using a sex-averaged genetic map instead of sex-specific maps for multipoint linkage analysis of affected sibling pairs when identity-by-descent states are incompletely known due to missing parental genotypes or incomplete marker heterozygosity. If either all or no parental genotypes were available, for intermarker distances of 10, 5 and 1 cM, we found no important differences in the expected maximum lod score (ELOD) or location estimates of the disease locus between analyses that used the best sex-averaged map and those that used the true sex-specific map for female:male genetic map distance ratios 1/10 or 10. In the extreme case of having genotypes for only one parental sex, the ELOD for the sex-averaged map was inflated compared to the ELOD for the sex-specific map if only mothers were genotyped and deflated if only fathers were genotyped for $r=1$ and intermarker distances of 5 and 10 cM. In addition, disease location estimates were biased in some cases for disease models with modest sibling relative risks. We are currently determining the extent to which less extreme cases of imbalances in missing parental genotypes influence the ELOD and location estimate. Preliminary results suggest that for larger sibships, the change in the ELOD and the bias in location estimate due to missing genotypes for one parental sex are attenuated. In summary, at the marker density used in most linkage studies, unless there is an imbalance in the number of mothers and fathers genotyped, using a sex-averaged map rather than a sex-specific map is unlikely to result in inflated lod scores, a loss of power, or the inability to localize disease susceptibility loci using affected sibships. If such an imbalance does exist, lod scores may be inflated or deflated, and location estimates may be biased.

Interactive visualization tools for genetic data. *M.W. Logue¹, J. Park², J. Ni^{2,3}, J. Cremer², A. Segre^{1,2}, B. Knosp³, S. Beck³, V. Vieland¹.* 1) CSGR; 2) Dept of CS; 3) AT, University of Iowa, Iowa City, IA.

The interpretation of complex genetic data analyses can be facilitated by interactive visualization tools. We have built two such tools, one for exploring high-dimensional genetic likelihood surfaces and one for exploring sampling distributions of alternative genetic statistics.

LiViT (Likelihood Visualization Tool) displays the HLOD (admixture LOD score) surface as a function of 6 input variables (3 penetrances, disease allele frequency, θ , and α) simultaneously. LiViT takes as its input a grid of pre-computed LOD score values and displays a 3D graph of the HLOD as a function of two of the model parameters. Which parameters are being displayed on the axes can be changed at any time. The values of the remaining explanatory variables are manipulated using slider bars. For example, the HLOD surface as a function of θ and α can be displayed at any arbitrary genetic model which can be changed in real time.

DiViT (Distribution Visualization Tool), is used to examine the null and alternative distribution of a statistic. DiViT takes as its input the cutpoint and bar heights which define two histograms. It outputs an applet which displays these histograms, representing the distributions of a statistic under the null and the alternative hypotheses. Slider bars manipulate color-coded regions superimposed on the distributions. The percentage of both distributions falling in each region is displayed to the right. Opening multiple DiViT windows allows comparison of statistics based on power, probability of false negative scores, as well as the probability of inconclusive results, for different size tests.

These graphical methods do not display any information which can not be displayed in a series of individual graphs. However, the interactivity enables the display of complicated information in a compressed form which is quickly understood in a clear and straightforward manor. In addition, use of the Java language has resulted in platform independent visualization tools leaving open the possibility of web-based versions which can be called from and/or integrated into a web site.

Program Nr: 2958 from the 2004 ASHG Annual Meeting

Power of the ordered subset analysis (OSA) method for linkage mapping in the presence of gene-environment interaction: Familial correlations and discrete vs. continuous covariates. *S. Schmidt, M. Schmidt, E.R. Martin, E.R. Hauser.* Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC.

The ordered subset analysis (OSA) method has recently been proposed as an approach to incorporate environmental covariates into parametric or non-parametric linkage analysis. The goal of the method is to identify more homogeneous subgroups of families on the basis of disease-associated covariates or phenotypic features, which may provide evidence for linkage that is otherwise obscured by genetic heterogeneity. When linked and unlinked families are distinguished by having different means of a normally distributed covariate with high familial correlations, the method was shown to have excellent power to detect linkage in the respective subgroup of families. Here, we present results of evaluating the performance of OSA when gene-environment (GxE) interaction exists at the individual level. We have incorporated exposure to a binary environmental covariate into the simulation algorithm implemented in the SIMLA program, which generates marker genotypes and binary disease phenotypes for families of fixed size given user-specified ascertainment criteria, parameters for a disease gene model, and a map of marker loci. We have assumed variable proportions of families linked to a disease gene that interacts with an environmental covariate. In the absence of familial correlations of the covariate, OSA has correct type I error but relatively low power to detect linkage in the presence of GxE interaction. The effect of familial correlations in environmental exposure (ranging from 0.1 to 0.9) and the benefits of using continuous rather than discrete (binary) covariates will be presented.

Program Nr: 2959 from the 2004 ASHG Annual Meeting

Robust Multipoint Identical-By-Descent Mapping with Affected Relative Pairs. *J.P. Sinnwell, D.J. Schaid, S.N. Thibodeau.* Biostatistics, Mayo Clinic, Rochester, MN.

The genetic mapping of complex traits has been challenging, requiring new statistical methods that are robust to misspecified models. Liang et al. [2001] proposed a robust multipoint method that can be used to simultaneously estimate the trait-locus position and its genetic effect, based on sib pair linkage data. We extend this approach to different types of affected relative pairs (ARP's) using two approaches. One approach is to estimate a common trait-locus position, while allowing different, unconstrained, trait-locus effects for different ARP types. This may be useful for older-onset diseases, where secular changes in diagnostic methods can change the frequency of phenocopies among different types of relative pairs. Our second approach also estimates a common trait-locus position, yet constrains the genetic effects to be consistent with a single susceptibility locus. Furthermore, we develop a robust score statistic to test whether there is significant evidence against the constrained model. Our methods are robust to genetic model assumptions and computationally efficient. These methods are applied to a prostate cancer linkage study, which emphasizes their potential advantages and limitations.

Relative Pair Inference: number of markers need to confirm pairwise relationships, use of family data to improve second degree relative inference, and impact of relationship misspecification on linkage analysis. *A.D. Skol, M. Boehnke.* Univ of Michigan, Ann Arbor, MI.

Relative pair inference is used to ensure that family structure is specified correctly prior to performing linkage or association analysis. We investigate three questions on this topic: 1) How many genotyped markers are necessary to be confident the putative relationship is correct? 2) How does using additional family members improve discrimination between second degree relatives? 3) How is evidence for linkage affected when second degree relatives are misspecified, for example, half sibs as uncle-nephew.

Our results suggest that genotypes for 60 well-spaced markers are sufficient for >80% power to identify putative full sibs(FS) as twins(MZ), parent-offspring(PO), avuncular(AV), grandparent-child (GG), half-sibs(HS), cousins(CO) or unrelated(UN), 30 are sufficient to identify putative PO pairs if their true relationship is among those listed above, and <20 are required for putative MZ twins. All other relative types we considered require >300 markers to achieve 80% power.

There is almost no power to discriminate between the three second degree relatives we considered; however substantial power can be achieved when additional sibs of the 'younger' relative are typed. For example, with ~90 well-spaced autosomal markers we have power .05 and .11 to detect that a putative AV pair is actually HS and GG. When an additional sib is added power increases to .74 and .82. Adding two sibs increases power to ~1. More modest improvements are observed for resolving HS and GG.

For all genetic models examined, regardless of the true second degree relationship, we found that AV pairs show the most evidence for linkage, followed by HS and GG pairs, implying that when either HS or GG are misspecified as AV there will be upward bias in the evidence for linkage and when AV and HS are misspecified as GG there will be downward bias. The magnitude of the bias appears most extreme when true AV relationships are misspecified, while misspecifying HS and GG pairs result in more modest bias.

Program Nr: 2961 from the 2004 ASHG Annual Meeting

Fast estimation of critical values of correlated genome scans for linkage. *S. Bacanu*. Dept Psychiatry, WPIC, Pittsburgh Medical Ctr, Pittsburgh, PA.

Estimates of study specific critical values for linkage scans (suggestive and significant thresholds) are important to identify promising regions. Often, scientists must perform multipoint linkage scans for several correlated traits or the same trait under different statistical models. Consequently, the outcome consists of several correlated multipoint linkage trace and many researchers chose to present the signal peaks from all traces, i.e. they report the performance of the pointwise maximal linkage trace. In this paper I propose a fast and concrete recipe for finding study specific critical values for the maximal linkage trace. Critical values could be derived theoretically or empirically. Theoretically-derived values are often conservative due to their assumption of fully informative transmissions. Empirically-derived values are computer and skill intensive and may not even be computationally feasible for large pedigrees. I propose a method to estimate critical values for correlated multipoint linkage traces using standard, widely used statistical software. The proposed method estimates study-specific critical values by using autoregressive models to estimate the correlation between standard normal statistics at adjacent map points for each trace and then use it, along with cross correlation with the other traces, to estimate study-specific critical values. The method is evaluated using different family structures and density of markers, under both the null hypothesis of no linkage and the alternative hypothesis of linkage between marker and disease locus. Overall, the proposed method appears very accurate in predicting critical values especially when the AR models are applied to the differenced time series.

Linkage tests for affected relative-pairs with marker IBD missing at random and marker IBS known. *D.W. Buckman, Z. Li.* Department of Statistics, The George Washington University, Washington, DC.

We develop linkage test statistics that utilize both the number of marker alleles shared IBD and the number shared IBS and allow the marker IBD value to be missing at random. These linkage statistics are developed for situations where the affected-relative-pair does not have other family members available to establish the number of marker alleles that the relative-pair share IBD. For each type of relative-pair considered, a likelihood function is obtained for this missing value situation. The linkage statistics for affected sib-pairs, affected aunt(uncle)-nephew(niece)-pairs, affected half-sib-pairs, and affected first-cousin-pairs are proportional to the second derivative of the log likelihood evaluated at the recombination fraction value of one-half. For affected grandparent-grandchild-pairs, the first derivative of the log likelihood is not fixed at zero when the recombination fraction equals one-half; therefore, the usual approach for developing an efficient score test is utilized. The asymptotic distribution of each linkage statistic is derived, and power and sample size equations are obtained. The linkage statistics are evaluated using simulated data based on various values for the recombination fraction, penetrances, and allele frequencies. Although the derivation is unique for each type of relative-pair, the same test statistic is obtained for affected grandparent-grandchild-pairs, affected aunt(uncle)-nephew(niece)-pairs, and affected half-sib-pairs. Under the simplifying assumption that the marker IBD is always known, the linkage statistics, based on the second derivative of the log likelihood, are members of the general set of linkage statistics derived by Dudoit and Speed (Dudoit, 1999; Dudoit and Speed, 2000). The power and sample size results reveal that discarding the affected relative-pairs with marker IBD missing at random is an inefficient strategy when compared to our more powerful approach that utilizes the marker IBS values for these relative-pairs. Also, the required sample sizes are smaller using our approach.

Sex-Specific Genetic Effects on BMI in the NHLBI FHS Study. *J. Corbett¹, I.B. Borecki¹, M.F. Feitosa¹, C.E. Lewis², K. North³, S.C. Hunt⁴, J. Hixson⁵, R.H. Myers⁶, D.K. Arnett⁷, M.A. Province¹.* 1) Div Biostatistics, Washington Univ, St Louis, MO; 2) Div Preventive Medicine, UA-Birmingham, Birmingham, AL; 3) School of Public Health, Univ of North Carolina, Chapel Hill, NC; 4) Dept Internal Med, Univ of Utah, Salt Lake City, UT; 5) School of Public Health, UT-Houston, Houston, TX; 6) School of Medicine, Boston Univ, Boston, MA; 7) School of Public Health, Univ of Minnesota, Minneapolis, MN.

Body Mass Index (BMI) is a primary phenotype used to study obesity. Some genetic linkage analyses of BMI have been performed using phenotypic information on only one sex. These analyses have yielded evidence for sex-specific genetic heterogeneity. We test for genotype by sex interaction in an analysis of 2,420 sib-pairs from the NHLBI Family Heart Study, a multi-center study to identify genetic and non-genetic factors that influence susceptibility to coronary heart disease. We use a Variance Components method for genetic linkage, implemented in the software package Mx, that allows for sex-specific genetic effects. The heritability of BMI is lower in females than in males ($p=0.05$). The estimated ratio of female to male heritabilities (estimated at 0.40 and 0.66 respectively) was 0.61 (0.39-0.96 95% CI). We also find evidence for a sex-specific differential in QTL effect size near 158 cM on chromosome 7 ($p=0.05$). These findings serve as a proof of concept for both the practicality and utility of studying sex-specific genetic effects in general, and provide additional valuable information in the study of the genetic etiology of obesity in particular. This methodology may be extended to incorporate other covariates for genetic linkage analysis in cases where there is reason to suspect genetic heterogeneity based upon that covariate.

Statistical properties of the propensity score as a single covariate in covariate-based linkage analysis. B.Q.

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To increase the power to detect linkage while minimizing the degrees of freedom of the linkage test, we previously introduced the first application of the propensity score (PS) to covariate-based linkage analysis (LODPAL). The PS is defined as the logistic regression of the affection status on the covariate data, thereby collapsing multiple covariate effects into a single variable. Through simulations of genetic models with two underlying covariate effects, we showed that in 68% of the models, the PS provided the highest power when compared to the inclusion of no, one or both covariates; and in the remaining 32%, the power was extremely low (<0.10) in all situations. The type I error rates increased approximately 20% with each increasing covariate analyzed. We have since examined the independent effects of increasing numbers of underlying covariates and of increasing sample sizes. With each additional covariate analyzed in the multiple covariate models, initial results suggest that the type I error rates continued to increase approximately 20%, with an average inflated type I error rate of 0.129 (0.05 nominal level) for 5 covariates analyzed. For the two-covariate models, increasing the sample size to 1000 families was sufficient to eliminate the inflation of the type I error rates, and with the additional power, the superior performance of the propensity score was then observed. In addition to determining the appropriate sample size for models with more than two underlying covariates, we are studying the statistical properties of the PS by verifying that the distribution of the covariates are similar between affected and unaffected individuals within strata defined by PS values (balancing property of the PS) and by comparing different definitions of pair-specific PS covariate values. These results will help determine the limitations of the PS as applied to covariate-based linkage analysis.

Identity-by-descent (IBD)-based variance component method to detect multiple genetic loci responsible for the complex diseases under a threshold model. A. Narita, A. Tajima, I. Inoue. Division of Genetic Diagnosis, Institute of Medical Science, University of Tokyo.

Many researches have indicated that multiple factors, either genetic or environmental, are involved in complex diseases, and thereby difficulties in the elucidation of the complicated architecture underlying these diseases are easily expected. We developed an effective tool to conduct identity-by-descent (IBD)-based variance component analysis to detect multiple genetic loci responsible for the complex diseases under a threshold model, in which a discrete trait is actually determined by a normally distributed variable, called liability, which depends on multiple genetic and environmental effects ($liability = + G + e$). Since the method uses the IBD matrices in which each element stands for the proportion of IBD alleles shared by two relatives, it can deal with loci with various number of alleles and modes of inheritance, and is not necessary to estimate haplotype frequencies for multiple loci. In addition, loci which have epistatic effects (gene-to-gene interactions) are also able to be taken into account. In the method, by combining the Bayesian approach via the Markov chain Monte Carlo (MCMC) algorithm, variance explained by each locus and its chromosomal location can be estimated simultaneously. Moreover, the reversible jump MCMC algorithm, which is a specialized version of the MCMC algorithm for the determination of the most likely genetic model through iterative additions and deletions of variables and has been hardly used in conventional methods, enables estimation of the number of loci responsible for the diseases as an unknown parameter. Effectiveness and flexibility of the method were demonstrated by using both simulated data and an actual clinical data set. The results indicate that some potential genetic loci, which have an important role in predispositions to these complex diseases but have not been found previously, will be additionally detected.

Variability in QTL Linkage Studies of Cross-Sectional Data. *G. Perez, M. Barmada.* Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA.

Power has been always of key concern in statistical human genetics. In cross-sectional cohorts, additional model parameters and complexities further complicate identification of genetic effects, and so reduce the power of the study. Longitudinal changes in genetic effects and the choice of analytic methodology might also influence the detection of genetic effects. We have undertaken a study to quantify the power of linkage studies of a quantitative trait by using simulated data from the GAW 13 study, which was simulated to resemble a population-based cohort study of cardiovascular disease. Ninety replicates were compared. Within each replicate longitudinal data was averaged to form a single observation, as is commonly done. Fasting glucose was selected as the trait of interest. Covariate effects were identified in one of three ways: (A) using data from founders; (B) using data from all individuals as if they were unrelated samples; or (C) using relationship-adjusted data from all individuals. Finally, residuals of the three methods were used for variance components genome-wide linkage. Differences were found within each replicate such as the number and type of covariates (varying randomly from A and B to C), LOD scores (which increased from A to B to C for values greater than 4), and the location of peak LOD scores (which varied erratically for lower values). A comparison between replicates shows that the selection of covariates varies widely confirming the difficulty of replication of a linkage study. The percentage of baseline genes (those involved in trait variation no matter what the patients age) detected also varied by autosome and position. For instance, for the baseline gene Gb13 (chromosome 9) the effects were detected 28% of the time (expected as our model did not agree with the one used in the study). Of this percentage, 28% of the time by using method A, 42% of the time by method B, and 100% of the time by method C. The highest rate of detection was for slope gene Gs3 (chromosome 5) that was detected 100% of the time (for all methods). By considering the result of the rest of the genes we are aiming at quantifying the loss of information as a way of assessing the accuracy of the current statistical methods.

A novel framework for sib pair linkage analysis. *G.D. Poznik¹, K. Adamska², X. Xu³, A.S. Krolewski¹, J.J. Rogus¹*. 1) Sect Genetics & Epidemiology, Joslin Diabetes Ctr, Boston, MA; 2) Institute of Informatics, Jagiellonian University, Cracow, Poland; 3) Dept Environmental Health, Harvard School of Public Health, Boston, MA.

Sib pair linkage analysis of a dichotomous trait is a popular method for narrowing the search for genes influencing complex diseases. While the pedigree structures are uncomplicated and the underlying genetic principles straightforward, there is a surprising degree of complexity involved in implementing a sib pair study and interpreting the results. Ascertainment may be based on affected, discordant, or unaffected sib pairs, as well as on pairs defined by threshold values for quantitative traits, such as extreme discordant sib pairs. In order to optimize power, various domain restrictions and null hypotheses have been proposed for each of these designs. Permutation of these factors and others yields a vast array of choices for the analyst. To begin, we systematically classify the major sources of discretion in sib pair linkage analysis. Then, we extend the work of Kruglyak and Lander (*Am J Hum Genet* 1995; 57:439-54) to bring the various forms into a unified framework and facilitate a more general approach to the analysis. Finally, we describe a new freely-available computer program, SPLAT (Sib Pair Linkage Analysis Testing), that can perform any sib pair statistical test currently in use, as well as any user-defined test yet to be proposed. In SPLAT, inheritance information is read once and stored in memory, while phenotype definitions can be modified at will, thereby providing an exceptional degree of flexibility for exploratory analysis. The program uses the EM algorithm (subject to user-specified conditions) to calculate maximum likelihood estimates of sharing and then plots the accompanying Lod scores versus chromosomal position. It also includes a novel grid scanning capability in which the entire Lod surface can be graphed for any position. This enables the simultaneous visualization of multiple test statistics, which can effect insight into both phenotype and genetics. The application of SPLAT will be illustrated with data from studies on the genetics of diabetic nephropathy.

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Using moderately discordant sib pair linkage analysis with ordinal diabetic nephropathy scores. *J.J. Rogus, G.D. Poznik, J.H. Warram, A.S. Krolewski.* Sect Genetics & Epidemiology, Joslin Diabetes Ctr, Boston, MA.

Nephropathy is a common complication of diabetes, and genes play an important role in susceptibility. The search for chromosomal regions harboring genes underlying diabetic nephropathy, however, has been complicated by a number of factors. First, mortality is high among those with diabetic nephropathy. Second, the trait is only of interest in the subpopulation of individuals with diabetes. Third, among this subpopulation, the sibling recurrence risk is particularly high (70%) relative to most other common diseases. For all of these reasons, affected sib pair analysis is an unattractive design. These obstacles were overcome with the advent of discordant sib pair analysis (Rogus and Krolewski; *Am J Hum Genet* 1996; 59:1376), which focused on sib pairs concordant for diabetes but discordant for nephropathy. One limitation of this design is its reliance on a dichotomous classification for nephropathy. To bring in additional information including duration and severity, we introduce an adaptation of the moderately discordant sib pair framework proposed by Forrest and Feingold (*Am J Hum Genet* 2000;66:1642). Specifically, we put forth an ordinal scale based on duration (above/below median) and renal status (e.g., normoalbuminuria, low/high microalbuminuria, proteinuria, end-stage renal disease) to move toward a more quantitative phenotype. This ordinal phenotype is then evaluated using both Haseman-Elston regression and allele sharing analysis, and the results are combined using a weighted composite statistic. We illustrate this procedure using data from a 10 cM scan for genes involved in nephropathy in type 2 diabetes (457 individuals with type 2 diabetes from 63 families).

An Alternative Paradigm For Statistical Evaluation of Linkage. *L.J. Strug, S.E. Hodge.* Div Stat Genetics, Dept Psych, Columbia Univ, NYSPI, New York, NY.

The lod score is central to linkage analysis and is traditionally translated into standard statistical terms such as p-values, -levels and power. However, an alternative statistical paradigm works directly with the lod or likelihood ratio (LR) (Royall, 1997, 2000), thus potentially resolving difficult issues of linkage criteria, multiple tests and low power. We applied this approach to linkage studies of a fully penetrant trait for: I. Fully informative gametes, II. Phase-unknown sib pairs, III. Nuclear families. The paradigm defines three types of evidence (whose probabilities sum to 1): strong (S), misleading (M), or weak (W), depending, respectively, on whether the observed LR exceeds k in the "right" direction, exceeds k in the "wrong" direction, or falls in between (k is a constant, e.g., 1000, corresponding to lod3.0). We calculated probabilities, $P[S]$, $P[M]$, and $P[W]$, exactly for situations I and II and via simulation for III, under no linkage and under linkage at recombination fraction $R=.01, .05, .1, .2, .3$; $k=8, 20, 32, 100, 1000$; and sample sizes $n=10, 20, \dots, 100$. $P[M]$ is analogous to type I error but has a natural upper bound ($1/k$) unrelated to R and n . The table shows $P[M]$ and $P[W]$, respectively, for situation III, for a dominant trait, testing $R=0.5$ vs. $R=0.1$. ($P[S]$, which we want to maximize, equals $1-P[M]-P[W]$.)

True R=0.5	n=10	n=20	n=30	True R=0.1	n=10	n=20	n=30
k=20	.006,.159	.003,.020	.000,.003	k=20	.004,.167	.000,.032	.000,.003
k=1000	.000,.554	.000,.113	.000,.019	k=1000	.000,.522	.000,.112	.000,.020

$P[M]$ remained small in all 3 situations (well below $1/k$). Larger k leads to lower $P[M]$ and $P[S]$ but higher $P[W]$. But as n increases, $P[W]$ and $P[M]$ converge to zero. The probability of concluding a false hypothesis is small for all n and gets smaller as n increases; thus, increasing n can adjust $P[M]$ for multiple tests. By controlling $P[W]$ with n , one can directly control the probability to detect correct linkage signals at any k .

Summarizing individual-level covariate information for covariate statistics for affected sib pair (ASP) linkage analysis. *H.-J. Tsai*^{1,2}, *D.E. Weeks*^{3,4}. 1) Lung Biology Center, San Francisco General Hospital, San Francisco, CA; 2) University of California, San Francisco, CA; 3) Human Genetics, University of Pittsburgh, Pittsburgh, PA; 4) Biostatistics, University of Pittsburgh, Pittsburgh, PA.

Genetic heterogeneity must be taken into account when geneticists search for disease genes in complex traits. Several statistical methods have been suggested for dealing with this issue by incorporating covariate information into ASP analysis. We have simulated three types of gene-environment (G x E) interaction models. We have evaluated the following covariate statistics: 1. mixture model (Devlin et al. 2002); 2. general conditional-logistic model-LODPAL (Olson 1999); 3. multinomial logistic regression-MLRM (Greenwood and Bull 1999); 4. maximum-likelihood-binomial approach-using Pearson's logistic regression residuals (Alcaï's and Abel 2001); 5. ordered subset analysis-OSA (Hauser et al. 2004); and 6. logistic regression for predicting IBD sharing probabilities-COVLINK (Saccone et al. 2001). Based on our findings, the mixture model and the OSA method perform the best of the covariate statistics when using a covariate with a G x E interaction effect.

We typically summarize individual-level covariate information by computing the mean covariate values across either the affected sib pairs or the whole family. However, it is not clear that taking the average is the best way to summarize individual-level covariate information. It is important to investigate what is the best summary for covariate information. Therefore, we applied the above covariate methods using alternative measures: taking difference of covariate values, summing the covariate values, using maximum covariate value, or using minimum covariate value. In our 8 disease models, covariate methods perform the worst when using the difference of covariate values. Individually, the mixture model is sensitive to various covariate measures and performs the best when using minimum covariate value. LODPAL has higher power when summing the covariate values. MLRM and OSA are robust to alternative covariate measures.

Recursive Partitioning Models for Affected Relative Pairs Linkage Analysis: Detecting Gene-Environment Interactions for Bipolar Disorder. *W. Xu*^{1,2}, *T. Schulze*³, *R. DePaulo*⁴, *F. McMahon*⁵, *C. Greenwood*^{1,2}. 1) University of Toronto, Canada; 2) Hospital for Sick Children, Canada; 3) Psychiatry Central Institute of Mental Health, Mannheim, GERMANY; 4) Johns Hopkins University, Baltimore; 5) National Institute of Mental Health, National Institutes of Health, Bethesda.

The interplay of environmental and genetic factors makes it difficult to distinguish the genetic effects from the background noise in phenotype variation. Here we present a novel approach that uses a recursive partitioning (RP) algorithm to search for covariates that influence linkage evidence. This method can be thought of as a data-mining approach for detecting gene-environment interactions for complex diseases. Standard concepts in RP models are modified to define the criteria for splitting as a function of the total likelihood ratio statistic for linkage in affected relative pairs across the covariate-defined subgroups. The model unifies the expressions for different types of relative pairs by using the same relative risk parameters for all relative types. The algorithm recursively splits each subgroup on pair-level covariates and cross-validation is used to choose the best tree-size at each marker. A bootstrap method is then used to get the best posterior tree-structure according to the chosen tree-size and to provide the final tree-structure for each marker. We applied this algorithm on data of 58 families from the Johns Hopkins/Dana bipolar pedigrees, for which linkage to chr. 18 has previously been reported. In this data set, we identified three suggested regions where covariates defined subgroups with stronger evidence for linkage than a standard linkage analysis. For some chromosomal locations, evidence of linkage was detected only when covariates were included. Covariates that showed strong interactions with genotype were age at onset, suicidal behavior, and alcohol/substance abuse. RP models may be a valuable tool to unveil gene-environment interactions. RP models can make use of different kind of relative pairs and a multitude of phenotypic variables at the same time.

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A likelihood ratio approach to family-based association and linkage studies with covariates. *M.F. Baksh, J. Whittaker, D. Balding, T. Vyse.* Faculty of Medicine, Imperial College London, United Kingdom.

We describe a procedure for analyzing genetic association and linkage using nuclear families that allows for dichotomous and more general measurements of phenotype and inclusion of covariate information. Standard generalized linear models are used to relate phenotype and its predictors. Our test procedure, based on the likelihood ratio, yields maximum likelihood estimates of the genetic relative risk and interaction parameters and it can be applied to complex scenarios, as for instance when multiple genes are involved. Our approach is compared with recently proposed conditional score tests that include covariate information in the underlying penetrance model and is shown to have advantages in coping with the baseline genotype risk, covariate and gene-covariate interaction parameters in the model. We apply our method in a study of human systemic lupus erythematosus and the C-reactive protein gene that includes sex and ethnicity together with interactions between these variables and the candidate locus.

Localization of linked genes for type 1 diabetes: A simultaneous search for two genes. *J.M. Biernacka*^{1,2}, *L. Sun*^{1,3}, *S.B. Bull*^{1,2}. 1) Department of Public Health Sciences, University of Toronto, Toronto, Canada; 2) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada; 3) The Hospital for Sick Children, Toronto, Canada.

Methods that consider multiple susceptibility genes may increase the accuracy and precision of location estimates for disease genes involved in the predisposition to complex disorders, such as type 1 diabetes. When two genes contributing to one trait are located near one another on the same chromosome, effects of both genes must be taken into account when assessing linkage evidence for one of them. Conditional methods are designed to localize a second disease gene locus and assess evidence of linkage to it, accounting for the effect of a pre-specified first gene locus (Farrall, 1997, *Genet Epidemiol* 14:103-115; Cordell et al., 2000, *Am J Hum Genet* 66:1273-1286), whereas simultaneous methods localize two putative disease gene loci at the same time (Biernacka et al., 2003, *Am J Hum Genet* 75(5S):193). The method of Biernacka et al. (2003) applies a generalized estimating equations approach to model IBD sharing data with multiple linked markers from ASPs. Confidence intervals for gene locations can be constructed based on large sample approximations, and test statistics can be calculated for evaluating evidence for two versus one disease genes in a single region (Biernacka and Bull, 2003, *Genet Epidemiol* 25(3):239). Here we apply these methods to data from a genome scan for type 1 diabetes (Mein et al., *Nature Genetics* 19: 297-300, 1998). We estimate the locations of two putative disease genes on chromosome 6, found to be approximately 20 cM apart. Our location estimates are similar to those obtained by applying a conditional approach to the same data (Cordell et al., 2000) partially because, in this case, the effect size of the first gene is predominantly larger than that of the second one. Our simulation studies of the GEE models indicate, however, that simultaneous estimation of two linked gene locations can yield more precise localization than estimating one location at a time.

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A classification method for pharmacogenetic surveillance. *C.E. Bowman.* Genetic Data Sciences, GlaxoSmithKline, Greenford, United Kingdom.

Using the practice of taxonomy as a model, a simple, sensitive, empirical diagnostic likelihood ratio-based framework is presented for the routine monitoring and detection of pharmacogenetic correlates of adverse events in pharmacovigilance. The supervised, sequential, multi-point, relative risk based classification method estimates and visualises the average objective positive evidence a reported individual gives in classifying adverse event cases as genetically different from controls. A bootstrap procedure is used to define a frequentist rule for stopping the collection of more cases. An example is given of its screening use against strong or weak signals comprised of binary patterns of large numbers of un-ordered single nucleotide polymorphisms (a high dimensional SNP profile) in real case control clinical pharmacogenetic data. Evidence for a possible genetic correlate of an adverse event may be apparent from the second case arriving. Counter-intuitively, only modest numbers of cases (less than 20) are needed, in the example used where a difference is present, to stop collection and claim such a correlate is worth investigating.

Quality check of the genealogical information in isolated populations using genetic data. *M. Ciullo¹, C. Bourgain², V. Colonna¹, A.L. Leutenegger², T. Natile¹, M.G. Persico¹.* 1) Institute of Genetics and Biophysics A. Buzzati Traverso, CNR, Naples, Italy; 2) INSERM U535, Villejuif, France.

Large pedigrees are a major advantage of isolated populations for complex trait mapping. This, in return, implies that quality of the genealogical information is a key issue for the success of these studies. However, classical approaches to validate simple relationships using genetic data are not adapted to the situation where individuals are related through multiple lines of descents. Furthermore, whereas only pairs of relatives may be informative to check the quality of the genealogy in outbred samples, inbred individuals alone may be informative. We have undertaken the genetic study of Campora population, an isolated village from the Cilento area of South Italy which dates back to the 17th century. The genealogy of the population has been carefully reconstructed using local church and town records allowing the identification of a 10,431-individual pedigree, spanning over 17 generations, and connecting all 1400 living individuals. A sample of 371 individuals, connected through a 2283-individual pedigree and with a mean inbreeding of 0.0058, has been genome screened with a 1094- microsatellite map (average marker distance : 3.4 cM, genotyping success rate : 89.6%). After classical checking for mendelian inconsistencies, we used two complementary approaches to evaluate the quality of our genealogical data. The EIBD approach proposed by Sun et al (2001) compares the pedigree-based kinship coefficient of a pair with its conditional expectation given the genetic data computed using both pedigree and genetic information. The method of Leutenegger et al (2003) only uses genetic data to estimate the inbreeding coefficient of an individual that may then be compared to its pedigree-based value. We present the results for each method and study their respective contribution in testing the accuracy of the Campora genealogy.

A locus on chromosome 13 for efficiency of real-word reading reveals complexities in ascertainment and genetic model selection. *R.P. Igo, N.H. Chapman, J.B. Thomson, M. Matsushita, Z. Brkanac, T. Holzman, M. Brown, V.W. Berninger, W.H. Raskind, E.M. Wijsman.* University of Washington, Seattle, WA.

Dyslexia is characterized by unexpected difficulty in learning to read. It has a strong genetic component but a complex mode of inheritance, with any of several specific cognitive deficits contributing to the disorder. Impairment in the efficiency and accuracy of reading single real words (word recognition, WR) is a strong predictor for dyslexia. We performed a whole-genome scan for two quantitative measures of WR: real-word reading efficiency (RWRE) and word identification (WID), which measure accuracy and speed, and accuracy alone, respectively. We describe a linkage signal on chr. 13 primarily involved in the efficiency of WR. We used variance components (VC) linkage analysis (LA), Markov chain Monte Carlo joint segregation and LA (MCMC), and parametric lod scores (pLOD) to assess linkage with a genome scan and phenotypes for 51 extended pedigrees ($n = 438$), plus only phenotypes on 57 additional pedigrees ($n = 436$). The measure of linkage for MCMC is the intensity ratio (IR), an estimate of the ratio of the posterior to prior probability of linkage. We obtained on chr. 13 two-point and multipoint VC lod scores for RWRE of 2.59 and 2.29, respectively, and an MCMC IR of 53.2. Adjusting RWRE for WID reduced but did not abolish the signal. We then obtained genetic models for RWRE, adjusted for age, sex and verbal IQ, by complex segregation analysis (CSA) and by MCMC. Using data from 108 families, the model obtained from MCMC yielded a greater max. pLOD (2.81) than did the model from CSA (2.19). The pLOD dropped when 127 additional families, ascertained under slightly different criteria, were included in segregation analyses (1.94 and 1.18 from MCMC and CSA, respectively). We attributed the differences in pLOD to differences in the ability to distinguish the heterozygous trait genotype from the major homozygote and to differences in the minor allele frequency. These comparisons show the importance of consistent criteria for ascertainment of pedigrees, and care in generating models by segregation analysis, in interpreting results from linkage studies. Supported by NIH HD33812.

Improved haplotype inference in case-control studies using genotype data. *L.C. Kwee*¹, *G.A. Satten*², *M.P. Epstein*³.
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Genetic association studies often use a case-control study design due to the ease of sample collection. Statistical methods that utilize prospective likelihoods for analyzing retrospective genetic data may suffer from a loss of statistical efficiency in certain situations, so it is of interest to use retrospective likelihoods when feasible. For haplotype analysis of case-control genotype data, Epstein and Satten (2003) proposed a retrospective likelihood approach that allowed for testing of both global and specific haplotype effects on disease. In Satten and Epstein (in press), they showed that their retrospective approach had optimal power for detecting haplotype-disease association relative to related prospective approaches. In the current work, we extend this powerful retrospective approach to allow for covariates, which permits us to model and test main environmental effects as well as gene-environment interaction effects. In order to accommodate the existence of ambiguous haplotypes in the genotype data, we apply a variant of the EM algorithm for proper inference. The power of this modified approach is compared to that of the prospective haplotype approach of Schaid *et al.* (2002). We also will apply our new method to case-control data from the Finland-United States Investigation of NIDDM (FUSION) genetic study.

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Strategies for uncovering the genetic basis of complex diseases. *J. Marchini*¹, *P. Donnelly*¹, *L. Cardon*². 1) Dept Statistics, Oxford Univ, Oxford, United Kingdom; 2) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, UK.

After nearly 10 years of intense academic and commercial research effort powerful genome-wide association studies are on the brink of reality. It is thus timely to discuss the fine details of study design and the relative merits of competing statistical techniques. The genetic basis of many complex diseases is likely to consist of interactions between unlinked loci. We have investigated strategies aimed at identifying the loci involved in such diseases within a genome-wide association framework. Specifically, we chose a set of realistic two locus models, fix their marginal effects at realistic levels and contrast a strategy that searches locus-by-locus against strategies that search more explicitly for interactions between pairs of loci. Very surprisingly, we find that direct interaction searches are more powerful than locus-by-locus strategies and are computationally tractable. These results should help to inform the design and analysis of genome-wide association studies. In addition, we provide a quantitative investigation of the assertion that allele frequency differences due to population structure provide a potential explanation for the lack of replication of some association studies.

Chip and cheerful? : The QTL problem of large sample sizes and large numbers of SNPs. *E. Meaburn, L. Butcher, L. Liu, C. Fernandes, R. Plomin, I. Craig, L. Schalkwyk.* SGDP, Institute of Psychiatry, London, UK.

The genetic influence found on complex traits is likely to be due to quantitative trait loci (QTLs) of small effect size. To detect QTL associations, large samples and systematic screens of thousands of DNA markers are needed, which is prohibitive in terms of time and cost. A solution is to genotype case and control DNA pools using SNP microarrays. We demonstrate that this is practical using pools of DNA from 100 individuals and the Affymetrix GeneChip Mapping 10K Array Xba 131. We constructed a control DNA pool independently three times, and assayed each on triplicate GeneChips. Each GeneChip gives two quasi-independent values for each SNP; RAS 1 & RAS 2. The average RAS was obtained for 108 SNPs located on the chip, and used to derive allele frequency estimates for the pooled DNA. These were compared to SNP allele frequencies determined by the individual genotyping of an independent Caucasian sample available from NetAffx Analysis Centre, and were found to correlate highly (0.901). They were also compared to SNP allele frequencies determined by genotyping of the 100 individuals used to construct the pool, and correlate even higher (0.94). This result indicates that RAS values can be used to provide valid quantitative measures of allele frequencies in pooled DNA. The correlations of RAS values within pool triplicates are very high (0.968 to 0.945; mean difference 0.059), as are correlations between pools (0.94 to 0.982; 0.058). 92.3% of the RAS values have a standard deviation 0.1, and 51.3% have a standard deviation 0.05. Using nine replicate chips per group would provide 98% power ($P=0.05$) to detect differences of 0.1. In addition we successfully tested the detection of allele frequency differences using reconstructed case pools spiked with 5, 10, 15 and 20% of the DNA of one genotyped individual. This innovative approach is rapid, cost effective, and accurate enough to screen the genome systematically in association studies of case and control DNA pools in order to nominate candidate QTLs for further study. We are currently applying the technique in two independent studies of Reading Disability & Mild Mental Impairment.

Confirmation of Linkage to a Genetic Susceptibility Locus on Chromosome 1 for Speech Sound Disorder and Language Impairment. *L.E. Sucheston¹, C.M. Stein¹, H.G. Taylor², L.D. Shriberg³, A. Kluge¹, C. Millard¹, K. Russo¹, N. Minich¹, A. Hansen², L.A. Freebairn², R.C. Elston¹, B.A. Lewis², S.K. Iyengar¹.* 1) Epidemiology and Biostatistics, Case Western Reserve Univ., Cleveland, OH; 2) Dept. of Pediatrics, Rainbow Babies & Childrens Hospital, Case Western Reserve Univ., Cleveland, OH; 3) Waisman Center on Mental Retardation & Human Development, University of Wisconsin-Madison, Madison, WI.

Despite the high prevalence of Speech Sound Disorder (SSD), not attributable to congenital malformations or developmental delay, the etiology has not been elucidated, although there is evidence from our previous work that dyslexia and SSD share genetic determinants (Stein et al. 2004). Specifically, dyslexia and language impairment (LI) have been shown to cosegregate in children with SSD, therefore we propose that other loci identified for dyslexia would also show linkage to SSD and LI. To test this hypothesis we examined the chromosome 1p34-36 region that was previously linked to dyslexia, specifically to single-word and phonological decoding (Grigorenko et al. 2001) and to spelling (Tzenova et al. 2004). We analyzed 10 markers spanning 78cM on chromosome 1, from D1S468 (4 cM pter) in 256 families with 367 sib pairs ascertained through a child with SSD. Using model-free linkage analysis we examined SSD, LI and Reading phenotypes. Parent-offspring allele transmission ($p=.03$) and affected sibling allele sharing ($p=.01$) for LI differed significantly from expectation at D1S199 and SSD affected IBD sharing showed significance proximal to D1S199 ($p<.03$). Using Haseman Elston regression, affection for SSD ($p=.02$) and LI ($p=.03$) showed linkage signals, as did quantitative measures of vocabulary (EOWPVT, $p=.015$; PPVT, $p=.03$), verbal short term memory (SI, $p=.01$), and word decoding (WRID, $p=.04$). Our data are the first to show that regions initially linked to dyslexia predispose to broader cognitive deficits including SSD and LI, suggesting that some segments of these disorders may be part of a broader continuum and serve to provide evidence for SSD susceptibility loci at 1p34-36.

Genetic Epidemiologic study of metabolic syndrome in a Chinese population. *Y. Feng¹, X. Hong², T. Zang², G. Tang², Z. Li², B. Wang¹, F. Hu³, L.J. Wei⁴, X. Xu^{1,2}, X. Xu¹.* 1) Prog Population Genetics, Harvard Sch Public Health, Boston, MA;; 2) Anhui Medical Univ. Institute of Medicine, Anhui, China;; 3) Nutrition Department, HSPH, Boston, MA;; 4) Biostatistic Department, HSPH, Boston, MA.

Metabolic syndrome (MS), a cluster of abdominal obesity, dyslipidemia, hyperinsulinemia, hyperglycemia and hypertension, is an important risk factor for cardiovascular disease. We have been conducting a large genetic epidemiologic study of MS in a rural community in Anhui, China since February 2004, with the ultimate goal of understanding the phenotypic structure of MS and both environmental risk factors and genetic components of MS. We'll screen 10,000 sibling pairs aged 40-64 years on traits related to MS, including BMI, fat mass, blood pressure, serum lipids, fasting plasma glucose and insulin. Meanwhile, information on lifestyle, diet intake, physical activity, and disease history are collected through a questionnaire. So far, we have enrolled a total of 5052 subjects aged 46.5+/-7.4 years from 1482 nuclear families. Preliminary analysis on the available data indicates the profiles of MS traits in this population differ significantly from that in US. The study population was quite lean with a mean BMI of 21.8 kg/m². According to Chinese criteria, 15.3% subjects were overweight ($24 \leq \text{BMI} < 28$) and 2.4% were obese ($\text{BMI} \geq 28$) in this population. By using the ATP-III criteria, the prevalence of MS was 7.9% in the study population, with a higher prevalence in women (10.6%) than in men (4.5%). The prevalence of individual component of MS was 0.3% of men and 4.3% of women with abdominal obesity; 14.3% of men and 23.4% of women with high triglyceride; 20.3% of men and 57.8% of women with low HDL; 35.9% of men and 27.4% of women with elevated blood pressure; and 8.9% of men and 10.6% of women with hyperglycemia, respectively. Our study suggests ATP-III is not appropriate as diagnosis criteria in the study population, and establishment of population specific criteria is warranted. When complete data is available in the near future, we will perform factor analysis to understand the structure of MS phenotypes in this population.

Impact of preferential mistyping of SNP heterozygotes on power to detect genetic association. *K.N. Conneely¹, P.S. Chines², M.R. Erdos², M. Boehnke¹*. 1) Dept. Biostatistics, U. Michigan, Ann Arbor, MI; 2) NHGRI, NIH, Bethesda, MD.

Low signal in one or both alleles in heterozygotes is a common source of genotyping error in SNPs. Homozygous genotypes are read as a single peak with a stronger signal and usually do not have this problem. In a sample of ~500,000 raw genotypes on 522 SNPs from the Sequenom Biomass genotyping system in the FUSION study that were ultimately resolved with confidence, 2% of heterozygotes were initially mistyped as homozygotes, while only 0.2% of homozygotes were mistyped. Mistyping is not evenly distributed. Of the mistyped heterozygotes, 83% were from plates that had >20% of heterozygotes mistyped. Heterozygotes are also more likely to be lost due to bad assays. 13% of initial assays of heterozygotes were inconclusive due to bad spectra or low probability calls compared to 10% of assays of homozygotes. We explore the impact of preferential mistyping of heterozygotes on the validity and power of association tests. The commonly used 2x2 test of equal allele frequencies between cases and controls is only a valid ² test under Hardy-Weinberg equilibrium (HWE). Under systematic mistyping of heterozygotes as homozygotes, this test is generally biased in favor of rejection, particularly for low allele frequencies. However, the test remains valid if heterozygotes are mistyped as either homozygote with equal probability. Random loss of heterozygotes from the sample also biases the 2x2 test in favor of rejection, especially for allele frequencies ~.5. The 2x3 test of equal genotype frequencies between cases and controls is robust to deviations from HWE. When heterozygotes are lost or mistyped, the 2x3 test loses power but remains a valid test. To ensure test validity and avoid loss of power due to genotyping error, it is common practice to retype plates for SNPs that deviate significantly from HWE, and those with substantial missing data. We are using our replicate genotype data to estimate the impact of redoing these assays and to build a model to determine when the cost of retyping is justified by the gains to validity and power. We also are assessing when statistical imputation of missing data based on linkage disequilibrium can eliminate the need for retyping.

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The RPM: A method for testing candidate QTL for epistatic interactions. *R. Culverhouse, T. Klein, W. Shannon.*
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Epistatic interactions play an important role in complex diseases: a few loci with little or no single-locus effects can nonetheless control phenotype to a large degree. The Restricted Partition Method (RPM) is an algorithm for examining multi-locus genotypes as predictors of quantitative traits in unrelated individuals. Tests in simulated and real data show that the RPM can provide an efficient way to identify loci contributing epistatically to a quantitative trait, even if the loci have no single locus effects (i.e. purely epistatic models). The RPM can also be used to detect gene-environment interactions.

Our earlier work showed that the RPM can identify contributing loci from purely epistatic 2-locus models when the allele frequencies for the contributing and null loci are all 0.5. Our current simulations applying the RPM to more complex models verified three important properties of the RPM: (1) the RPM is robust to variations in allele frequencies at both the contributing loci and at null candidate loci, (2) the RPM is computationally feasible and has good statistical power for the analysis of 3-locus models, and (3) two-locus analyses of 3-locus models can have good power to identify contributing loci.

In an application of the RPM, a 2-locus analysis of leukemia data identified an interaction between genes GSTP and UGT1A1 in Caucasians explaining 29% of the variation in etoposide (a chemotherapy agent) area under the concentration vs. time curve ($p=0.036$) and an interaction between GSTP and sex in African Americans explaining 62% of the variation in etoposide clearance ($p=0.018$). None of these covariates were significant univariate predictors of trait variation.

Conclusion: The RPM is robust under variations in allele frequencies (e.g. good power, nominal false positive rates), practical for examining 2-way and 3-way interactions, and performs as expected in real data.

Power comparison of the Regression of Offspring on Mid-Parent (ROMP) method and Quantitative trait Transmission - Disequilibrium Tests (QTDT). *M.-H. Roy-Gagnon, R.A. Mathias, A.F. Wilson.* Genometrics Section, IDRB, NHGRI, NIH, Baltimore, MD.

The Regression of Offspring on Mid-Parent (ROMP) method is an extension of the linear regression of offspring on mid-parent traditionally used to estimate the heritability of a quantitative trait. ROMP also provides a test of association between the trait and a marker locus and an estimate of the heritability attributable to that locus. ROMP requires parent-offspring trios with phenotype data on both parents and offspring but genotype data on the offspring only. Transmission Disequilibrium Tests for quantitative traits (QTDT) require genotype data on both parents and offspring. Depending on the feasibility and cost of phenotyping compared to genotyping, ROMP may provide an efficient and cost effective alternative to QTDT. The goal of this study was to perform simulation experiments to compare the power of ROMP to that of QTDT.

ROMP was compared to two QTDT statistics implemented in the QTDT software: Allison's TDTQ5 and the orthogonal model of Abecasis et al. (2000). Power at the 5% significance level was estimated from 2000 simulated samples of 200 parent-offspring trios with complete phenotype and genotype data. The trait was determined in part by up to 3 biallelic loci with locus-specific heritabilities ranging from 5 to 30%. Simulations were also performed for a trait under no genetic control to investigate type I error rates. The allele responsible for an increase in the trait value had a frequency of 0.3, yielding an average of 132 trios with informative genotypes for QTDT analysis. The 200 trios were informative for ROMP but the ROMP analysis was performed only on 132 trios selected at random to obtain equal sample size on average for the two methods. Estimated type I error rates were close to the 5% nominal value for both methods. For a locus-specific heritability of 5%, the estimated power of ROMP was close to 70% on average while the power of QTDT was 62%. Both methods had over 90% power to detect locus-specific heritabilities of 10% or greater. Overall, the estimated power of ROMP was similar to, or higher than that of QTDT.

A Genotype-based Transmission/Disequilibrium Test for Tightly Linked Markers. *T. Wang, Q. Qing, R.C. Elston.*
Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH.

The transmission/disequilibrium test (TDT), which can be robust to population stratification, is a popular method to detect disease liability alleles on the basis of family data. Because only the families in which at least one parent is heterozygous are informative for transmission, in most cases extended marker haplotypes are more informative than single markers. However, there is a trade off when choosing between extended marker haplotypes and single markers: the number of degrees of freedom of the test versus the information about transmission. The degrees of freedom of a TDT test based on haplotypes increase rapidly with the number of markers and therefore may reduce the power of the test. Furthermore, the haplotypes of parents may be ambiguous and, in general, the proportion of families with ambiguous haplotypes increases with the number of markers. To avoid loss of information because of ambiguous families, the haplotypes have to be reconstructed probabilistically based on statistical inference. The potential gain in efficiency from using haplotype inference is still debatable. Here, we develop a TDT-type statistic, called gd-TDT, for tightly linked markers based on the relationship between a trait and genotype distance. The genotype distance carries information about each marker and the pair-wise linkage disequilibrium among markers. The degrees of freedom of this statistic increases only linearly with the number of markers. It is not necessary to infer haplotypes in order to calculate our statistic. The statistic can be used for nuclear pedigrees of any size, and for both quantitative and discrete traits. The performance of the gd-TDT, in terms of type I error rate and power, is examined by computer simulations.

Twin and Sibling Pair Designs for Genetic Association Studies. *J. Wessel*^{1, 2, 3}, *A.J. Schork*⁴, *N.J. Schork*¹. 1) Polymorphism Research Laboratory, Department of Psychiatry, UC, San Diego, La Jolla, CA; 2) Department of Family and Preventive Medicine, UC, San Diego, La Jolla, CA; 3) School of Public Health, San Diego State University, San Diego, CA; 4) Undergraduate Studies Program, University of California at Santa Barbara, Santa Barbara, CA.

Twins have a long and illustrious history in human genetics research; for example, exploring evidence for the overall influence of genetic factors on a trait and disease. In addition, requisite statistical analysis tools for assessing heritability and related issues have been steadily improved over the years to the point that there is no shortage of methodology a researcher can choose from. However, one area where twin analyses have not received much attention involves genetic association studies. We consider novel twin and sibling pair-based study designs for dissecting the genetic basis of quantitative traits, derive formulae for computing power, discuss computer programs for assessing these equations, and showcase the utility of our derivations on a few select study settings. The increase in power can be substantial in certain settings. In general, a smaller sample of unrelated individuals is needed compared to twins. However, it can be shown that due to the correlations that exist between monozygotic and dizygotic twin pairs, well-designed studies using samples of genotypically discordant twins investigating the impact of allelic variation at a particular polymorphic locus on a quantitative trait are more powerful than samples of unrelated individuals taken from the general population. Furthermore, DZs with opposite genotypes can substantially decrease the required sample size, particularly when DZs are sampled in equal genotype proportions. Our results are easily extended to non-twin siblings. The fact that well designed twin studies can reduce association study sample sizes combined with the fact that one can use twins to simultaneously evaluate the effects of polymorphic loci, effects of loci merely linked to a marker locus, gross polygenic background effects, and measured and unmeasured environmental covariate effects, makes the use of twins for association studies particularly flexible and compelling.

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A sequential method on case-control study. *W. Yang*¹, *N. Kamatani*². 1) Genome Diversity Team, JBIRC, Tokyo, Japan; 2) Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan.

From an experimental point of view, one may wish to avoid unnecessary experimentation. Sequential analysis of quantitative data has never found wide application in practice, even though considering its use might be worthwhile. In the present study we propose, "sequential method", in which the sample size is not fixed in advance. We focus on the case of cohort case-control study that evaluate the lower and upper bound of the sample size based on the significant level and the power of test. Hypothesis testing in sequential method usually relates to testing for the numerical value of some unknown parameter, so we discuss it on case-control observations that the minimal sample size for detecting a true difference between case and control observations. We compare the effect of sequential method with the results of a non-sequential analysis.

Linkage Disequilibrium Mapping by the Decay of Haplotype Sharing with Samples of Related Individuals. *J. Zhang*¹, *D. Schneider*², *C. Ober*², *M.S. McPeck*^{1,2}. 1) Dept of Statistics, Univ of Chicago, Chicago, IL; 2) Dept of Human Genetics, Univ of Chicago, Chicago, IL.

We consider the problem of linkage disequilibrium mapping of a susceptibility locus for a complex trait from samples of related individuals. We work within the decay of haplotype sharing framework proposed by McPeck and Strahs (1999). For populations with dependent recombinational histories, we take a more flexible model than the conditional coalescence they considered by allowing for varying conditional correlation coefficients of ancestral-segment sharing for pairs of haplotypes in the sample. We describe how the conditional correlations can be calculated via a recursive algorithm in the case of a known 13-generation, 1623-member Hutterite pedigree. From the conditional correlations and a general estimating equation based on the marginal score functions, we obtain an approximated log-likelihood of the sample, which can be used for mapping purposes. We also make the extension of our method to trio genotype data using a hidden Markov chain formulation. The method is studied in simulated data on the basis of the Hutterite pedigree and we apply it to the problem of mapping a susceptibility locus for bronchial hyperresponsiveness (BHR) in the Hutterites. Reference: McPeck, M.S. and Strahs, A. (1999) Assessment of Linkage Disequilibrium by the Decay of Haplotype Sharing, with Application to Fine-scale Genetic Mapping. *Am. J. Hum. genet.* 65:858-875. Supported by NIH grants HG001645, DK55889, HL56399 and HL66533.

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Linkage Disequilibrium Mapping Using Block Haplotype Structure. *M. Zheng, M.S. McPeck.* Department of Statistics, University of Chicago, Chicago, IL.

We are interested in LD mapping using high-resolution haplotype block structure. For complete haplotype data, we can calculate a profile likelihood for each SNP or haplotype block and a confidence set can be constructed based on this profile likelihood. For (unphased) genotype data, the profile likelihood will not have the simple form that depends only on the data at the locus considered. Instead, a parsimonious model for background LD that actually fit for the data will be required. We tried to model the background LD by a class of Markov models where the time index is each block instead of each SNP. A parametric bootstrap method is used to assess the significance of the goodness of fit to our background LD models. We found that the traditional parametric bootstrap method to assess the goodness of fit of models does not have the right type I error in our case, where we have multinomial-type models with many cells having very low probabilities and only a moderate sample size. Error model were introduced to model the similarities between different haplotypes and to get the right type I error. We applied our method to Daly et al (2001) dataset where our confidence set covered the causal SNPs which were suggested in Rioux et al (2001). This work is supported by NIH grants HG001645 and DK55889.

Large-scale single-nucleotide polymorphism (SNP) and haplotype analyses of 199 drug-related genes in 752 subjects: Majority of uncommon SNPs within haplotype blocks were assigned to single haplotypes constructed with haplotype-tagging SNPs. *N. Kamatani¹, A. Sekine², T. Kitamoto², A. Iida², S. Saito², A. Kogame¹, E. Inoue¹, M. Kawamoto¹, M. Harigai¹, Y. Nakamura².* 1) Division of Genomic Medicine, Department of Advanced Biomedical Engineering and Science, Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan; 2) Laboratory of Genotyping, RIKEN SNP Research Center, Riken Yokohama Institute, Yokohama, Japan.

We extensively analyzed single-nucleotide polymorphisms (SNPs) and haplotypes in 199 drug-related genes using 4,190 SNPs in 752 control subjects to optimize the strategies for population-based pharmacogenetic studies. Drug-related genes, like other genes, have a haplotype-block structure, and a few haplotype-tagging SNPs (htSNPs) could represent most of the major haplotypes constructed with common SNPs in a block. Based on such data, we analyzed the relationship between haplotypes and uncommon (minor allele frequency 0.1) SNPs within the blocks (549 SNPs). We inferred haplotype frequencies using the data from all htSNPs and one of the uncommon SNPs within a block and calculated four joint probabilities concerning the haplotypes. We show that, irrespective of the minor allele frequency of an uncommon SNP, the majority (mean SD, 0.943 0.117) of the minor alleles were assigned to a single haplotype tagged by htSNPs if the uncommon SNP was within the block. These results support the hypothesis that recombinations occur only infrequently within blocks. The proportion of a single haplotype tagged by htSNPs to which the minor alleles of an uncommon SNP were assigned was positively correlated with the minor allele frequency when it was 0.03 (P 0.000001). The results of simulation studies suggested that haplotype analysis using htSNPs may be useful in the detection of uncommon SNPs associated with phenotypes if the frequencies of the SNPs are higher in affected than in control populations, the SNPs are within the blocks, and the frequencies of the SNPs are 0.03.

Use of recursive partitioning to identify genetic marker combinations associated with adverse events. *M. Mosteller, D. Zaykin, L. Warren, D. Yarnall, W. Spreen, T. Scott, A. Nelsen, K. Nangle, E. Lai, J. Hernandez, S. Haneline, A. Gatherum, K. Davies, A. Hughes.* GlaxoSmithKline, RTP, NC.

Abacavir (ABC) is an effective antiretroviral drug used to treat HIV-1 infection. Approximately 5% of patients treated with ABC develop a hypersensitivity reaction (HSR) that in rare cases has proved fatal. The ABC clinical risk management program has been successful in reducing serious outcomes due to ABC HSR. Performance characteristics for a prognostic ABC HSR marker set must be extremely high in order to improve upon clinical management; over-reliance on prognostic markers for ABC HSR could lead to reduced clinical vigilance and more serious outcomes for patients who do not carry the predictive markers. Thirty-eight markers were associated ($p < 0.05$) with HSR in Caucasians ($n \sim 1000$) in two retrospective case-control studies. Among these replicated markers, HLA-B*5701 had the highest performance characteristics: sensitivity=56.4%, specificity=99.1%. HLA-B*5701 was not associated with HSR in Blacks. These data imply that Caucasians who test positive for HLA-B*5701 would have a 73.0% risk of HSR while those who test negative would have 2.3% risk of HSR. Recursive Partitioning (RP), a data mining method that detects interactions within large sets of predictor variables, was used to investigate marker combinations predictive of HSR risk. Application of RP led to the identification of genotype combinations involving multiple single nucleotide polymorphisms (SNPs). For example, one RP algorithm comprised of 6 SNPs assigned patients to one of five risk categories. If this algorithm was prospectively validated and applied to a random population of ABC-treated patients, it is estimated that 19% of patients would be assigned to a risk group for which the HSR risk is estimated to be zero, 35% would be in 1-2% risk group, 38% in the 3-4% group, 4% in the 5-50% group, and 4% in the 50-100% group. Despite uncertainty around the clinical utility of markers identified to date for ABC HSR, RP may be a useful tool for the identification of genetic marker combinations that predict safety and/or efficacy of medicines.

Pairwise analysis of genetic marker combinations predictive of an adverse drug reaction. *L. Warren¹, D. Zaykin¹, K. Davies¹, D. Yarnall¹, A. Handley¹, S. Haneline¹, A. Hughes¹, E. Lai¹, K. Nangle¹, T. Scott¹, W. Spreen¹, A. Wooster².* 1) GlaxoSmithKline, RTP, NC; 2) Five Points Consulting, RTP, NC.

Abacavir (ABC) is an effective antiretroviral drug used to treat HIV-1 infection. Approximately 5% of patients treated with ABC develop a hypersensitivity reaction (HSR) that in rare cases has proved fatal. The ABC clinical risk management program has been successful in reducing serious outcomes due to ABC HSR. Performance characteristics for a prognostic ABC HSR marker set must be extremely high in order to improve upon clinical management; over-reliance on prognostic markers for ABC HSR could lead to reduced clinical vigilance and more serious outcomes for patients who do not carry the predictive markers. Thirty-eight markers were associated ($p < 0.05$) with HSR in Caucasians ($n \sim 1000$) in two retrospective case-control studies. Among these replicated markers, HLA-B*5701 possessed the highest performance characteristics: sensitivity=56.4%, specificity=99.1%. While specificity, the probability of being negative for HLA-B*5701 among individuals who did not develop HSR, was quite high, sensitivity was only moderate (e.g., about 44% of Caucasian patients who developed HSR were negative for HLA-B*5701). Thus, the goal was to improve upon the sensitivity of HLA-B*5701 while maintaining its high specificity. An algorithm was developed to systematically examine genotype combinations for all 703 marker pairs among the 38 replicated markers. This algorithm revealed marker pairs that were highly associated with HSR. 97% of the 703 pairs were significant at $p < 0.05$, 27% at $p < 10^{-10}$, and 4% at $p < 10^{-30}$. In 47% of pairs ($n=331$), sensitivity was increased compared to the contributing markers and in the remaining 53% of pairs ($n=372$) specificity increased. No pair exhibited increases in both sensitivity and specificity and no combination resulted in performance characteristics substantially superior to those of HLA-B*5701 alone. Despite uncertainty around the clinical utility of markers identified to date for ABC HSR, this analytical approach may contribute to the identification of marker pairs predictive of adverse drug reactions.

Quantifying intra-familial phenotypic variation for hereditary hearing loss: a tool for the identification of modifier genes. *E. Fransen¹, E. Van Eyken¹, L. Van Laer¹, P.L.M. Huygen², G. Van Camp¹*. 1) Department of Medical Genetics, University of Antwerp, Antwerp, Belgium; 2) Department of Otorhinolaryngology, University Medical Centre St Radboud, Nijmegen, the Netherlands.

We have previously studied five families with monogenic late-onset nonsyndromal hearing loss due to mutations in the KCNQ4 gene. This gene encodes a potassium channel involved in the K⁺ recycling pathway in the inner ear. Audiologic analysis of the families with a KCNQ4 mutation has shown a correlation between the mutation and the audiological phenotype: Four families with a missense mutation showed progressive hearing loss starting in the high frequencies, gradually affecting all frequencies at a similar annual threshold shift. In one family with a truncating mutation, only high-frequency HL was observed, while hearing in the low frequencies was conserved (De Leenheer et al. (2002) *Adv. Otolaryngol.* 61:41-46). The severity of the phenotype varied considerable within each of the five families, which may be due to genetic or environmental modifying factors. Here we have developed a quantitative measure that describes how severely a person is affected, compared to the other family members and correcting for age. We refer to this measure as the family-specific Z-score. To build a family-based Z-score, we first regressed the audiometric thresholds at each frequency on age, which showed a linear relationship between threshold and age at each frequency. Different regression equations were calculated for each family and for each frequency. For each patient, we calculated the expected hearing thresholds and the residual (observed minus expected). Hereby, a negative residual means a better hearing than expected. This residual was converted into a Z-score by dividing by the standard error of the estimate of the regression analysis. This family-based Z-score was squared, but the sign (+/-) was kept. Hence, a least-square correction for the trait as a whole is obtained. This method opens the perspective for genetic studies aiming at the identification of modifier genes for different forms of genetic hearing impairment, or even for other quantifiable monogenic disorders.

Univariate and Multivariate Genetic Analysis of Migraine Symptom Data. *D.R. Nyholt, N.G. Martin.* Genetic Epidemiology Lab, QIMR, Brisbane, QLD, Australia.

Familial typical migraine is a common, complex disorder that shows strong familial aggregation. Studies indicate that migraine affects up to 25% of females compared with 7.5% of males in Western populations.

Migraine shows both clinical and genetic heterogeneity. The exact cause is unknown and there are no recognisable, diagnostic, pathological changes. Therefore, the diagnosis of migraine is largely based on the retrospective reporting of headache characteristics and groupings of their associated symptoms. In 1988, the International Headache Society (IHS) prepared a new classification for headaches, which has made diagnosis clearer and more precisely defined. However, it is *not* clear how the diagnostic components are related both to each other and to the familial aggregation of migraine.

A twin study design was used to examine the extent of genetic influence on the expression of IHS symptoms of migraine. The symptoms were investigated using heritability as an indicator of validity. A total of 2662 (42.5%) twins screened positive for migraine or recurrent headaches.

Results show how one of the four major IHS criterion shows no heritability, and therefore should be either revised or ignored. Moreover, only 6 out of the 10 IHS criteria examined, showed significant heritability ranging from 0.26 (unilateral location) to 0.63 (moderate or severe intensity). These individual symptom heritability results will allow optimisation of the phenotype definition in molecular genetic studies of familial typical migraine, while investigation of individual clinical criteria may lead to the genetic dissection of specific migraine symptoms.

Although results from multivariate genetic analyses indicate two sets of genes are typically inherited together, they do not however indicate migraine with aura is genetically distinct from migraine without aura. These results have immediate application to both presently collected pedigree data and also to the future design of molecular genetic studies of familial typical migraine.

Semistructurate Phenotypification of Patients with ADHD in the Colombian Population. *H.M. Velasco¹, A. Julio¹, E. Espinosa², G. Casas⁴, A. Fajardo², S. Guerrero², N. Mancilla², J. Mongui⁵, P. Mancera³, C. Duran¹, J.C. Prieto^{1,3}.*
1) Instituto de Genetica Humana, Universidad Javeriana, Bogota, Cundinamarca, Colombia; 2) Universidad Militar, Bogota, Colombia; 3) Hospital la Victoria, Bogota, Colombia; 4) Instituto Franklin Delano Roosevelt, Bogota, Colombia; 5) Clinica Psiquiatrica San Juan de Dios, Manizales, Colombia.

The ADHD has a frequency in Colombia from the 8 to 18% and it is the more frequent psychiatric diagnosis infantile population. A prospective study of ADHD positive patients without mental retardation was made to characterize them in its different subtypes, comorbidity, medications response and antecedents until third degree by means of criteria of DMS IV, semistructurate interview and valuation by paediatric neurology, child psychiatry and clinical genetic using software designed for such aim. 78 patients (75.64% men and 24.36% women) were finding with ages between the 4 to 15 years evaluated themselves ($X=8.29$ $DS=2.22$). 50% of them were combined ADHD, impulsive hyperactive 19.23%, 19.23% inattentive and non-specific 11.54%. 23% did not have any comorbidity, 33% have one comorbidity and 44% have more than one comorbidity, being the most frequent oppositional defiant disorder (72%) and the of anxiety disorder (28%). 75% took some medicine and 25% were treaty by means of support therapies. The percentage of good response to the treatment was of 51.3%, to regulate 14%, none responded of 25.5%. and it does not know of 9%. 18.5% of the patients had some relative with a neurological disease, a 60.5% with psychiatric antecedents and a 17% with both. Significant differences with other similar studies in the variables of low socioeconomic layer, impulsive subtype inattentive and hyperactivity, subset oppositional defiant disorder comorbidity and depression were observed. In Conclusion the epidemiological variables were similar with other populations, however in our population the subtype inattentive and hiperactivity-impulsive were more prevalent than others countries.

ApoE gene polymorphisms in three ethnic groups living in Turkey. *G. Akpınar, E. Ergül, A. Sazci.* Department of Medical Biology & Genetics, , Faculty of Medicine, University of Kocaeli, Kocaeli, Turkey.

The ApoE gene is a polymorphic gene located at chromosome 19q13.2 with three common alleles, epsilon 2, 3, and 4, producing three isoforms of the protein E2, E3, and E4. These isoforms differ in amino acid sequence at positions 112 and 158. From these alleles arise six genotypes; ApoE3/3, E3/4, E2/3, E4/4, E2/2. To investigate the genotypes and allele frequencies of ApoE gene in three different ethnic groups in Turkey, we analysed DNA isolated from peripheral blood samples using PCR-RFLP method and digesting by HhaI restriction endonuclease, and followed by statistical analysis. The genotype frequencies in Turkey of the Bulgarian immigrants (N=136; 54 male (39.7%), 82 female (60.3%); mean age= 46.8plusminus13.6 years) with ApoE2/2, E2/3, E2/4, E3/3, E3/4, and E4/4 were 0.7%, 16.2%, 5.1%, 65.4%, 11.8%, and 0.7; respectively. The allele frequencies of the Bulgarian immigrants of Apo e2, e3, e4 were 11.40%, 79.41%, and 9.19. The genotype frequencies of the Tunceli community (N=142; 74 male (52.1%), 68 female (47.9%); mean age= 37plusminus13.56 years) with ApoE2/2, E2/3, E2/4, E3/3, E3/4, E4/4 were 9.9%, 1.4%, 77.5%, 10.6%, and 0.7; respectively. The allele frequencies of Apoe2, e3, e4 of the Tunceli community were 5.63%, 87.68%, and 6.69; respectively. The genotype frequencies of the Georgian immigrants (N=124; 55 male (44.4%), 69 female (55.6%); mean age= 49 plusminus15.67 years) living around Ordu with APOE2/2, E2/3, E2/4, E3/3, E3/4, E4/4 were 0.8%, 12.9%, 2.4%, 68.5%, 15.3; respectively. The allele frequencies of Apoe2, e3, e4 of the Ordu community were 8.47%, 82.66%, and 8.87; respectively. In conclusion, the allele frequencies of e2 ranged from 5.63% in the Tunceli community to 11.40 % among the Bulgarian immigrants. On the other hand, the allele frequencies of e3 ranged from 79.41 % in the Bulgarian immigrants to 87.68% in the Tunceli community. The allele frequencies of e4 ranged from 6.69% in the Tunceli community to 9.19% in the Bulgarian immigrants. Thus, three different ethnic groups studied in Turkey demonstrated significant variations in the three different alleles of ApoE gene.

Val-9Ala polymorphism in the manganese superoxide dismutase(MnSOD) gene is associated with increased risk of Coronary artery disease in the Korean obese male. *E.Y Cho¹, H. Cho², S. Kim^{1,3}, J.E. Lee^{1,3}, Y. Ko^{1,4}, J.H. Lee^{1,5}, H. Park^{1,4}, Y. Jang^{1,4,5}.* 1) Cardiovascular genome center, Cardiovascular Research Institute, Yonsei University, Seoul, Korea; 2) Research Institute of Science for Aging, Yonsei University; 3) DNA link, Inc, Korea; 4) Division of Cardiology, Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Korea; 5) Department of Food and Nutrition, College of Human Ecology, Yonsei University, Seoul, Korea.

The Oxidative stress contribute to atherogenesis and manganese superoxide dismutase(Mn SOD) on the mitochondria are catalyzing dismutation of reactive superoxide radicals to hydrogen peroxide. The purpose of this study was evaluated the role of genetic variation in the MnSOD gene on CAD in Korean. 148 male patients with CAD and 263 healthy male controls were participated in this study. Among the study subjects 83 controls were analyzed for serum SOD activity and lipid peroxidation as measured by Malondialdehyde(MDA). The genotype was determined by SNP IT assay. The genotype frequencies of the MnSOD for Val/Val, Val/Ala, Ala/Ala were 81, 47 and 3% for control and 73.6, 25.7 and 0.7% for CAD, respectively. Overall, Odds ratio(OR : 1.40, 95% CI :0.81-2.41) were not significantly elevated in Ala carrier compared to Val/Val genotype. However, the frequencies of Ala allele(0.19 vs 0.08) and Ala carrier (15.6% vs 36.4%, $p=0.004$) of the MnSOD gene were significantly higher in obese CAD patients than in obese controls. In the multivariate logistic model as covariate with age, plasma levels of total cholesterol, triglyceride, insulin resistance index, smoking and drinking status, Ala allele was an independent risk factor for CAD(OR :3.11, 95% CI:1.29-7.71, $p<0.05$). MnSOD Val-9Ala polymorphism was not associated with severity of CAD and presences of Myocardial infarction. Mean SOD activity and MDA concentration were not significantly different between the Ala carrier and Val/Val genotype. It is concluded that the Val-9Ala polymorphism of the MnSOD gene is not associated with SOD activity and MDA, but is a genetic risk marker for obese CAD Korean.

MTHFR gene polymorphisms in four ethnic groups in Turkey. *E. Ergul, G. Akpınar, A. Sazci.* Department of Medical Biology & Genetics, Faculty of Medicine, Kocaeli University, Kocaeli, Turkey.

We investigated the allele and genotype frequencies of the MTHFR C677T and A1298C polymorphisms of four ethnic groups in Turkey using a PCR-RFLP method. The five ethnic groups studied were Bulgarian immigrants of Turkish origin (N=136; 54 male (39.7%), 82 female (60.3%); mean age= 46.8±13.6 years), Bosnian immigrant (N=110; 54 male (49.09%), 56 female (50.91%), turkmen from Horasan (N=101; 32 male (31.7%), 69 female (68.3%), mean age=50.08±15.9), Tunceli community (N=142; 74 male (52.1%), 68 female (47.9%); mean age= 37.13±5.6 years) living in Turkey. The genotype frequencies of the MTHFR C677C, C677T, and T677T of the Bulgarian immigrant were 46.3%, 46.3, and 7.4 respectively. The allele frequencies of MTHFR 677T and 677C were 30.51%, and 69.49% respectively. The genotype frequencies of the MTHFR A1298A, A1298C, and C1298C were 42.6%, 50.7, and 6.6 respectively. The allele frequencies of MTHFR 1298A and 1298C were 68.01%, and 31.99% respectively. The genotype frequencies of MTHFR C677C, C677T, and T677T of the Tunceli Community were 55.6%, 36.6, and 11 respectively. The allele frequencies of MTHFR 677C and 677T were 73.96% and 26.06% respectively. The genotype frequencies of MTHFR A1298A, A1298C, and C1298C were 30.3%, 49.3, and 20.4 respectively. The allele frequencies of MTHFR 1298A and 1298C were 54.93% and 45.07% respectively. The genotype frequencies of MTHFR C677C, C677T, and T677T of the Turkmen community were 31.7%, 52.5, and 15.8 respectively. The allele frequencies of MTHFR 677C and 677T were 67.92% and 42.08 % respectively. The genotype frequencies of MTHFR A1298A, A1298C, and C1298C were 61.4, 35.6, 3% respectively. The allele frequencies of MTHFR 1298A and 1298C were 79.21%, and 20.79% respectively. The genotype frequencies of MTHFR C677C, C677T, T677T of the Bosnian community were 37.3%, 46.4, and 16.4 respectively. The allele frequencies of MTHFR 677C and 677T were 60.45%, and 39.55% respectively. The genotype frequencies of MTHFR A1298A, A1298C, C1298C were 44.5%, 42.7, and 12.7 respectively. The allele frequencies of MTHFR 1298A and 1298C were 65.91%, and 34.09% respectively.

Additive effects of Interleukin-1B, -RN and -10 risk alleles on susceptibility for hip osteoarthritis. *I. Meulenbelt¹, J. Min¹, A. Seymour², H.A.P. Pols³, C.M. van Duijn³, P.E. Slagboom¹.* 1) Molecular Epidemiology, Leiden Univ Med Ctr, Leiden, Netherlands; 2) Pharmacogenomics and Antibacterials, Inflammation & Immunology Pfizer Global Research & Development, Croton, USA; 3) Epidemiology & Biostatistics Erasmus Univ Med School, Rotterdam Netherlands.

Osteoarthritis (OA) is characterized by degradation of articular cartilage and formation of new bone. Identification of genes that influence disease susceptibility may indicate which pathways may be involved in the onset of OA. Matrix integrity is maintained by the chondrocyte by a delicate balance between the effects of catabolic cytokines IL1 and TNF, which induce production of specific matrix degrading metalloproteases and the anabolic cytokines IL10, IL4, and IL1Ra that have been shown to prevent and reverse cartilage degradation. A failure to maintain the balance between pro- and anti-inflammatory cytokines may result in cartilage degradation. Previously the -511T allele in the promoter of the IL1B gene and allele 2 of the VNTR in the IL1RN gene were found to be independently associated with the occurrence of radiographic OA (ROA) in the hip in a random sample of 55-65 year old subjects from a population-based cohort (The Rotterdam study, N = 809). In this sample ROA has been assessed in knee, hip, hand and spine. One interesting promoter SNP in the IL10 gene is the G-2849A since the A allele was consistently negatively associated to *ex vivo* IL10 production. Here we show that this SNP was also associated to hip ROA (OR is 1.5, 95% CIL 0.9-2.7 and 2.2, 95% CIL 0.9-5.0 for respectively hetero- and homozygous carriers of allele A). An increasing risk was observed for hip ROA among carriers of increasing number of risk alleles of the C-511T, VNTR and G-2848A polymorphisms (OR is 2.0, 95% CIL 0.6-7.6 for carriers of 1 risk allele, 4.4, 95% CIL 1.3-15.2 for carriers of 2 risk alleles, 7.3, 95% CIL 2.1-25.4 for carriers of 3 risk allele and 5.8, 95% CIL 1.4-22.8 for carriers of 4 risk alleles). Together these data implicate that different alleles within the IL1B, IL1RN and IL10 genes independently and additively contribute to the susceptibility for hip ROA.

Program Nr: 3000 from the 2004 ASHG Annual Meeting

Association of CD 14 promoter polymorphism with Asthma in African Americans. *K. Yanamandra, P.B. Boggs, D. Napper, S.A. Ursin, H. Chen, R. Dhanireddy, J.A. Bocchini Jr., T.F. Thurmon.* Dept Pediatrics, LSU Medical Ctr, Shreveport, LA.

Allergic diseases such as asthma are multifactorial disorders with polygenic and environmental combinations in their etiology. Many allergic diseases have increased IgE levels in their etiology, which in turn, is under strong genetic control. Several studies have repeatedly reported association between 5q31-33 (loci containing Interleukin-4, Interleukin-5, Interleukin-13, CD 14 genes and others) and total serum IgE level and its dependent disorders such as asthma. Although many of these studies were conducted in Caucasians, there were limited studies in the African Americans. Our laboratory studies on interleukins has shown a strong association between IL-13 promoter polymorphism and asthma for the first time in African Americans. We also showed a strong association of IL-13 Arg130Gln (A398G in coding region) polymorphism with asthma in this community. Also, we showed that in the IL-4 receptor coding region (A+398G), mutant 398G allele frequency was significantly higher (0.31 vs 0.18) in patients as compared to controls. In the present investigation we have extended our asthma research to study the association of CD 14 promoter polymorphism (C-159T) in seventy-nine asthma patients and one hundred sixty controls of African American ancestry. We used microplate PCR-RFLP method with Ava II restriction enzyme. The restriction products revealed a frequency of 0.089, 0.081 of TT-159 homozygotes in patients and controls respectively. The CT-159 heterozygote frequency and CC-159 normal homozygote frequencies were 0.392, 0.059 in patients and 0.425, 0.494 in controls respectively. Our results do not show any difference in the allele frequencies of either 398C or 398T between the controls and asthma patients (398T frequency was 0.29 in patients and 0.29 in controls) in contrast to the published literature from the Caucasians. This being the first report on CD 14 polymorphism analysis in African Americans, experience from other researchers is needed to verify the validity of these initial findings. Data and statistics will be presented.

Relationship between Endometriosis and Polymorphisms of Detoxification Enzymes NAT2, GSTM1 and CYP 1A1 in the Korean female population. *H.W. Choi¹, H.-S. Lee¹, H.J. Song², S.Y. Park³, M.K. Koong², J.H. Jun¹.* 1) Infertility Research Lab., Samsung Cheil Hospital, Seoul, Korea; 2) Dept. of Ob/Gyn, Samsung Cheil Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea; 3) Lab. of Medical Genetics, Samsung Cheil Hospital, Seoul, Korea.

Endometriosis is a common gynecologic disorder and inherited as a complex genetic trait, related to multiple genes interacting with each other and with the environmental factors. This study was performed to evaluate the relationship between endometriosis and polymorphisms of detoxification enzymes N-acetyl transferase 2 (NAT2), glutathione S-transferase M1 (GSTM1), and cytochrome P450 (CYP) 1A1 in the Korean female population. By laparoscopic diagnosis, they were classified into Group-I (minimal/mild endometriosis, n=166) and Group-II (moderate/severe endometriosis, n=85) and Controls (without endometriosis, n=98). DNA was extracted and amplified using PCR. The genotypes of each gene were examined with restriction fragment length polymorphism (RFLP) analysis. The frequencies of NAT2 fast-acetylation genotypes and GSTM1 active genotypes in Group-II were higher than those of Controls, respectively (91.8% vs 87.8%, p=0.5196; 58.8% vs 44.9%, p=0.0834). The frequencies of the CYP1A1 MspI polymorphism (62.4% vs 59.2%) and of the combination of the GSTM1 null mutation and the CYP1A1 MspI polymorphism (65.7% vs 64.8%) were similar between Group-II and Controls. We found that the NAT2 *4/*4, *4/*6 and *4/*7 genotypes were common in the Korean female population whereas the NAT2 *4/*5, *5/*5 and *5/*6 genotypes in the Caucasian and European population. These discrepancies between our results and the other reports could arise because of ethnic variations in allele frequency. Based on our results, detoxification enzymes, NAT2, GSTM1 and CYP1A1, might be indirectly associated with the risk for endometriosis. More studies are needed on the other detoxification systems and interactions with environmental factors for the etiology of endometriosis.

Program Nr: 3002 from the 2004 ASHG Annual Meeting

Androgen receptor and endometrial cancer risk. *I. De Vivo, M. McGrath, S.E. Hankinson, P. Kraft, D.J. Hunter.*
Dept Medicine, Channing Lab, Harvard Medical School, Boston, MA.

The androgen receptor (AR) gene is a transcription factor responsible for mediating the physiological effects of androgens. Evidence suggests that androgens and its receptor are involved in uterine cell proliferation, thus potentially affecting endometrial cancer risk. A polymorphic CAG repeat in exon 1 of the AR gene encodes a polyglutamine tract that is inversely correlated with the transcriptional activity of this gene. We assessed the association between the functional CAG repeat polymorphism and the risk of endometrial cancer in a nested case-control study within the Nurses Health Study (n = 222 cases, 666 controls). Women with one or more long AR CAG repeat alleles (> 22 repeats) were at a decreased risk of endometrial cancer (odds ratio (OR) = 0.69; 95% confidence interval (CI), 0.46-1.02). Women with one or two long alleles (> 27 repeats) compared to both alleles <22 repeats were also at a decreased risk (OR= 0.52; 95% CI, 0.27-1.01). We observed an association between average repeat length and endometrial cancer risk (OR= 0.91; 95% CI, 0.84-0.99). Additionally, six haplotype-tag polymorphisms (htSNPs) have been determined to predict the common haplotypes in the AR gene for Caucasians. We genotyped these htSNPs in our nested endometrial case-control study and found no significant associations between the haplotypes and endometrial cancer risk. Our findings suggest that the functional CAG repeat in the AR gene may be involved in endometrial carcinogenesis by possibly downregulating its target gene IGF-1.

Heritability of obesity-related traits among multigenerational Afro-Caribbean pedigrees. *I. Miljkovic-Gacic¹, C.M. Kammerer¹, X.J. Wang¹, M. Kenney¹, V. Wheeler², A.L. Patrick², J.A. Cauley¹, C.H. Bunker¹, J.M. Zmuda¹.* 1) Un. of Pittsburgh, Pittsburgh, PA; 2) Tobago Health Studies Office, Scarborough, Tobago.

Obesity, a global public health problem that has reached epidemic proportions, contributes to the development of some of the most prevalent chronic diseases in modern society. There is considerable evidence for a genetic liability to obesity and its associated phenotypes, but very little data regarding the genetics of obesity in populations of African heritage. We present preliminary results from an ongoing initiative to unravel the genetic and environmental contributions to obesity phenotypes within multigenerational Afro-Caribbean families. All family members undergo a comprehensive body composition evaluation including dual-energy X-ray absorptiometry (DEXA) assessments of total and regional body composition and quantitative computed tomography measures of muscle cross-sectional area, and subcutaneous and intermuscular fat. Weight, standing and seated height, and body mass index (BMI) are measured with standardized protocols. The heritability of DEXA phenotypes and the effects of age and gender were estimated using quantitative genetic methods and preliminary data collected on 128 individuals (43% male) aged 18 to 86 years (average age 43 years) from 7 extended pedigrees. Age and gender explained 45% of the variance of FFM, 34% of TBF, 58% of %fat and 25% of TF within these pedigrees. The DEXA derived measures of non-bone soft tissue mass (FFM), total body fat (TBF), percentage body fat (%fat) and trunk fat (TF) all showed significant residual heritability (h^2 S.E.): 0.440.23; 0.410.22; 0.760.16; and 0.370.26, respectively. In contrast, the heritability of BMI was considerably lower (0.140.14) than the heritabilities of the DEXA traits. These results suggest that FFM, TBF, TF and particularly %fat are highly heritable traits in these Afro-Caribbean pedigrees and that these DEXA phenotypes may be more informative for genetic association and linkage studies than the BMI, the more traditional index of obesity. Further analyses of these DEXA phenotypes should greatly increase our understanding of the genetic susceptibility to obesity and its related complications in populations of African descent.

Admixture Association in a Systemic Lupus Erythematosus Puerto Rican Population. *A.I. Quintero-Del-Rio*^{1,2}, *C.E. Aston*¹, *X. Mao*⁵, *D.C. Hutchings*¹, *J. Kilpatrick*¹, *A. Santiago-Cornier*³, *J.B. Harley*^{1,2,4}, *M. Shriver*⁵. 1) Oklahoma Medical Research Foundation, Oklahoma City, OK; 2) Oklahoma University Medical Center, Oklahoma City, OK; 3) Ponce School of Medicine, Ponce, PR; 4) Department of Veterans Affairs Medical Center, Oklahoma City, OK; 5) Pennsylvania State University, University Park, PA.

Admixture studies provide a means in isolated, admixed populations to localize disease susceptibility genes using approximately 100 times fewer markers than are required for whole-genome scans. Systemic lupus erythematosus (SLE) is a serious autoimmune disease with strong genetic component affecting people of all ethnic backgrounds, especially minority groups. The native population of Puerto Rico experienced a bottleneck after Western contact with as few as 2500 founders. Immigration of Europeans and Africans into the island has increased the Puerto Rican population to 4 million people today compose of 18% Taino Indians, 45% European and 37% African. Thus, the Puerto Rican population is ideal for an admixture study of SLE susceptible genes. We performed a genetic association study specifically seeking the effects predicted by population isolation and admixture. Using single nucleotide polymorphism analysis and pooling techniques in 43 Puerto Rican lupus patients and 38 controls, we observed powerful association and nucleotide variation in different chromosomes. These association included chromosomes 1, 2, 10, 13, and 18 from $p < 10^{-5}$. Ancestral informative markers were typed in the same group of Puerto Ricans and 59 genes on 14 chromosomes demonstrated significant differences in admixture proportions between cases and controls. These genes are mainly located in chromosomes 1, 2, 3, 10, 16 and 18. The preliminary results of this study draw upon the key advantage of admixture mapping that is based on directly associating sections of the genome with disease. This study provides insight in to a population not previously described and may have more power than linkage to detect the presence of modest effects not previously identified using the variants that differ strikingly in frequency across the three ancestral populations of the Puerto Ricans.

Addressing the multiple testing problem in detecting gene-gene interaction by a permutation-based method. *N. Wang, R. Chakraborty.* Ctr Genome Information, Univ Cincinnati, Cincinnati, OH.

Identification of the genetic and environmental contributions to human diseases is one of the primary goals of biomedical research. For human common complex diseases, such as cancer, heart disease, asthma, and diabetes, etc, there may be more than one underlying gene responsible for the causation, making the investigation complicated. Multiple testing is one of the potential problems in reporting a gene-gene interaction related to certain disease in population based case-control studies. In order to avoid the type I error (false positive), Bonferroni correction has been applied in many studies. However, markers of different genes under study may not be truly independent (e.g., they may exhibit linkage disequilibrium), making the adjustment too conservative. With this motivation, we developed a novel method to deal with the multiple testing problem. Given a specific genotype combination, we calculated the traditional χ^2 first, considering the sampling scheme (say, case-control study design). Next, we shuffled each marker genotype across case and control individuals with a large number of replication (say, 10,000 times), and calculated the χ^2 value of the specific genotype combination for each replicate. We assigned the preliminary significant level for the specific genotype combination as the relative frequency with which we observed χ^2 values from replicates equal to, or higher than the χ^2 from the original data. Then we applied Bonferroni correction to determine the final significant level. With the preliminary significant level being obtained from random shuffling of markers, this step helps us ensure that the follow-up Bonferroni correction is based on the assumption of independent testing. Gene-gene interactions being very common underlying many human complex diseases, the method we present may be one of the better ways to deal with the multiple testing problem that control reporting false positive results. (Research supported by NIH grant GM 41399).

Determination of population structure in the Mayo Alzheimers Disease Patient Registry. *S. Melquist¹, R. Crook¹, B. Phillips¹, J. Gass¹, J. Adamson¹, R. Mueller², R. Petersen², M. Hutton¹.* 1) Department of Neuroscience, Mayo Clinic College of Medicine, Jacksonville, FL; 2) Alzheimer Disease Research Center, Mayo Clinic College of Medicine, Rochester, MN.

Although traditional linkage analysis has identified late-onset Alzheimers disease (LOAD) candidate gene loci, no definitive risk variants (apart from APOE-e4) have been isolated. Alternative approaches, such as population-based linkage disequilibrium (LD) mapping, might be more effective in identification of risk variants. However, concerns exist regarding the validity of these types of studies, because many LOAD gene variants identified by this approach have not been replicated in subsequent studies using alternate populations. One explanation for this lack of replication may be that underlying population structure difference between case and control subjects is a confounding factor in the original studies. The Mayo Alzheimers Disease Patient Registry (ADPR), consisting of 293 case and 448 control subjects collected from Olmstead County, Minnesota, is potentially a rich resource for genome-wide LD mapping. We hypothesized that population structure may exist in the ADPR samples and underlying structure would be a confounding factor in future LOAD LD studies using this series. Therefore, we examined the extent of population structure in the ADPR series by genotyping a number of unlinked single nucleotide polymorphism and short tandem repeat markers and analyzing the genotype data using the program *structure* developed by Pritchard and colleagues. The results of the structure analysis were consistent with two major subpopulations in the ADPR. Case and control subjects were equally distributed between the two populations. These results may reflect the predominant Scandinavian origin of many individuals in this region, along with a second more general US population. We used population structure information in the series when fine mapping LOAD candidate loci. Results from these analyses will be presented.

Program Nr: 3007 from the 2004 ASHG Annual Meeting

Application of an Ancestry-Informative SNP Panel to a Large Cosmopolitan Sample. *M.S. Pungliya, B.A. Salisbury, J.C. Stephens.* Population Genomics, Genaisance Pharmaceuticals, New Haven, CT.

A genomic control panel of 38 SNPs was selected from over 100,000 candidates discovered through resequencing 79 unrelated diverse humans. The goal in creating the panel was to have an efficient tool for estimating the geographic ancestry of new subjects. The SNPs were chosen to distinguish African, European, Asian, and Native American components of ancestry. To test the utility of the panel, we applied it to a set of 256 unrelated subjects, most of whom indicated uniform ancestry from one country. The genotype data for these new subjects were submitted to the program Structure 2.0. Under the parameter setting of five populations, the program found evidence for distinct East Asian, African, and Native American ancestries. In addition, it recognized an Indo-European spectrum spanning Europe, the Indian subcontinent and the lands in between. Several outlier subjects were notable. Subjects from the Caribbean had the widest mix of ancestral proportions. Latin Americans were also quite diverse; while many of the individuals were estimated to have over 90% Native American ancestry, others were marked as having nearly none. The ancestral Native American population seems clearly characterized both by unique SNPs and by SNPs that are otherwise characteristic of East Asians. This study supports the use of carefully selected SNP panels for efficient assessment of study samples.

BIASES DUE TO POPULATION STRATIFICATION IN EPIDEMIOLOGICAL STUDIES OF GENE-GENE INTERACTIONS. *Y. Wang, R. Localio, T. Rebbeck.* Department of Biostatistics and Epidemiology University of Pennsylvania School of Medicine 904 Blockley Hall, 423 Guardian Drive Philadelphia, PA 19104-6021.

It has been widely recognized that Population stratification (PS) can result in spurious disease-gene associations in epidemiological studies, yet its roles in gene-gene interaction studies remain unfamiliar. We used logistic regression models to fit multiplicative interactions between two genes for a binary disease outcome in a hypothetical cohort of two ethnicities. Biases on main effects and interactions due to PS were evaluated by comparing regression coefficients in mis-specified models that ignored ethnicities with their counterparts in models that accounted for ethnicities. We revealed that PS could exert more bias to interaction estimates than main effects estimates. Biases in main effects and interaction estimates were bounded by the log-odds ratio and two times the log-odds ratio of disease risks in the two unobserved ethnicities, respectively. Potential biases from PS in gene-gene interaction studies were evaluated under a wide range of conditions. Biases in interaction estimates were most serious when two genes were under linkage disequilibrium, even when marginal distributions of both genes were same across ethnicities. This may be a particular concern in the studies of multiple SNPs in adjacent chromosome regions in substructured populations.

Propensity Score methods to adjust for population stratification in association studies. *E. Ziv*¹, *S. Choudhry*^{1,2}, *E. Burchard*^{1,2}. 1) Department of Medicine, University of California, San Francisco; 2) Lung Biology Center, University of California, San Francisco.

Population stratification may lead to an increased risk of false positive and false negative results in case-control studies. Several methods have been developed to adjust for population stratification in case-control studies using information from additional unlinked markers. Here we propose an alternative approach based on the concept of propensity score methods in classical epidemiology. The propensity score is a method of reducing a large number of potential confounders to one. The score is derived based on the association between the confounders and the main predictor. In a genetic association study, the propensity of having a particular genotype at the locus being tested is modeled based on the association between that genotype and other unlinked markers. The propensity of having the genotype being tested can then be compared among cases and controls. A significant difference between cases and controls implies that the association being tested is confounded by the difference between the genetic background of the cases and controls. The propensity scores can also be used in the context of model-based approaches such as logistic regression to adjust for confounding. We test the method on an existing dataset of 44 ancestry informative markers tested in a case-control study of asthma in Puerto Ricans. Without adjustment for stratification 7 of 44 markers are significantly associated with asthma. After adjustment for propensity only 2 of 44 markers are significantly associated, a rate that is consistent with usual type I error. The method may easily be applied to quantitative phenotypes. We propose that the propensity approach may be an efficient and straightforward approach to adjust for population stratification in association studies.

Modeling stochastic effects in gene regulation in eukaryotes. *P. Paszek*¹, *T. Lipniacki*^{1,2,3}, *A.R. Brasier*⁴, *M. Kimmel*^{1,5}. 1) Department of Statistics, Rice University, Houston, TX; 2) Institute of Fundamental Technological Research, Warsaw, Poland; 3) Bioinformatic Program, University of Texas Medical Branch, Galveston, TX; 4) Department of Internal Medicine, University of Texas Medical Branch, Galveston, TX; 5) Institute of Automation, Silesian Technical University, Gliwice, Poland.

Transcriptional gene enhancement and regulation is a stochastic phenomenon due to a small number of the molecules involved. Activity of a gene can be initiated by a single trans-activator molecule bound to the specific regulatory site in the targeted gene. In eukaryotes, the gene activation results in production of bursts of mRNA transcripts, hence the average mRNA level is relatively large (order of tens or hundreds of molecules). The stochasticity of activator binding and dissociation to and from the promotory region of targeted gene is then amplified by translation, since each copy of mRNA serves as a template for numerous protein molecules. In prokaryotes, the stochastic effects are attributable primarily to erratic transcription, translation and degradation of mRNA and its products. This is due to low abundance of mRNA transcript molecules (several molecules per cell), which causes that production or degradation of just one mRNA transcript molecule has significant effect on cell behavior. In the present work, we briefly discuss various stochastic effects in gene expression, and then focus on regulation of gene activity in eukaryotes. We introduce a mathematical description of the stochastic effects embedded in the ODEs framework assuming the binary nature of gene activation; at a given time a gene is switched either on or off, depending on whether trans-activator molecules are bound to the gene promoter or not. Also we develop complementary representation of the model in terms of PDEs describing probability density functions of gene being in active or inactive state. We compare and illustrate the two modeling approaches with an example of a single auto-repressive gene.

Evaluation of genetic tests for asthma susceptibility with receiver operating characteristic (ROC) curves. Y.

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Susceptibility to a complex genetic disease such as bronchial asthma is not determined by a single gene but by a set of genes. To assess genetic risk of an individual, integration of many polymorphisms of susceptibility gene may be needed. Recently, Yang et al. (*Am J Hum Genet* 72: 636-649, 2003) argued that disease prediction for multifactorial diseases was greatly improved by considering multiple genetic polymorphisms with a likelihood ratio method. With this approach, they showed that three genetic polymorphisms could express 8 fold risk difference in venous thrombosis. Janssens et al., however, criticized this approach showing the low prevalence of high-risk combinations of genes, and recommended use of the standard method to evaluation of discriminative ability of a test, namely, evaluation by sensitivity and specificity (*Am J Hum Genet* 74:585-588, 2004). The changes in sensitivity and specificity of a test under different cut-off points can be overviewed by the receiver operating characteristic (ROC) curve. The area under ROC curve (AUC) indicates the discriminative ability of a test. We evaluated the usefulness of genetic tests with multiple candidate genes for asthma in the Japanese population with AUC. Use of C3, ADRB2, IL4RA, and CYSLT2R gene polymorphisms in a logistic regression model resulted in AUC of 0.83. This value was better than the values (0.76-0.77) for discrimination of patients and controls by lung functions but worse than that (0.91) by exhaled nitric oxide (Malmberg *LP Thorax* 58:494-499, 2003). The ability of the current multi-gene test for asthma seems to be compatible with conventional clinical tests. By updating susceptibility gene polymorphisms, we can expect better AUC values of genetic tests for asthma.

MAOA: A Primary Candidate Gene of Persistent, Pervasive Childhood Aggression. *S. Ehtesham, H. Mik, L. Douglas, J. Kennedy, J. Beitchman.* Neurogenetics, CAMH, Toronto, Ontario, Canada.

Monoamine oxidase A (*MAOA*) expression has a direct regulatory impact on the serotonin, norepinephrine, and dopamine systems, each of which have been implicated in the etiology of aggression. Previous studies have demonstrated that a functionally relevant variable number of tandem repeats (VNTR) in the promoter region of *MAOA* is associated, directly¹ or indirectly², with aggression in humans. In the present study, we examined the relationship between the two polymorphisms, *MAOA* VNTR and the single nucleotide polymorphism (SNP) at codon 297 and persistent, pervasive Childhood-Onset Aggression (COA).

Our clinical sample of aggressive children consisted of 57 psychiatrically referred males (mean age 9.72.6). All clinical subjects were rated above the 90th percentile on the Aggression subscale of both the Child Behaviour Checklist (mean t score 68.6013.93) and the Teacher's Report Form (mean t score 67.8513.94), with a minimum of 2-year history of aggressive behaviour. Ethnically matched adult males with no history of psychiatric illness served as controls (n=57).

Almost three-quarters of the COA sample carried the high-transcription four repeat allele (n=40, 70.2%), compared to half of the adult controls (n=28, 49.1%) in the VNTR ($\chi^2=5.25$; d.f.=1; p=0.02). In the codon 297 SNP, there was no significant difference in the allele frequencies of the two samples ($\chi^2=0.038$; d.f.=1; p=0.85). The *MAOA* VNTR and codon 297 polymorphisms were in moderate linkage disequilibrium ($D'=-0.61$).

Our findings suggest that higher transcription levels of the *MAOA* is associated with the persistent, pervasive COA. This is consistent with reports on dispositional aggressiveness among adults¹, but differs from reported² findings regarding an interaction between childhood maltreatment and the *MAOA* high- and low-transcription alleles as predictors of adult antisocial behaviour.

1. *Manuk et al.* Psychiatry Res 2002, 95:9-23.
2. *Caspi et al.* Science 2002, 297:851-854.

Age of onset for Huntington's disease with results in the range 36-39 repeats. *O. Quarrell^{1,3}, A.S. Rigby², A. Dalton³, Y. Crow³, A.E. Fryer³, F. Heydon³, E. Kinning³, A. Lashwood³, M. Losekoot⁴, L. Margerison³, S. McDonnell³, P.J. Morrison³, A. Norman³, M. Peterson³, F.L. Raymond³, S. Simpson³, J. Warner³.* 1) Dept Clinical Genetics, Sheffield Children's Hosp, Sheffield, United Kingdom; 2) Academic Cardiology, University of Hull, Hull, UK; 3) UK Huntington's Disease Consortium, UK; 4) Stichting Klinisch Genetisch Centrum, Leiden, Netherlands.

Following a genetic test for Huntington's disease (HD), a result of 36-39 repeats is considered abnormal; reduced penetrance may occur; however, the data on the degree of reduced penetrance are limited. Ethical approval was obtained for a multi-centre study to collect data anonymously on age of onset, or age last known to be unaffected, from a large cohort of individuals with such results. Wherever possible, DNA was re-analysed in a reference laboratory; Kaplan Meier analysis was used to construct an age of onset curve and penetrance figures. To date, 138 cases have been included in the analysis; of these, 119 had results concordant with the referring centre; of the remainder, DNA was not available or re-analysis failed. The median age of diagnosis was 54 years (range 31-91 years). The number of cases with 36, 37, 38 and 39 repeats was 24, 30, 30 and 54 respectively. There was no statistical difference between the age of onset curves based on individual repeat sizes, or combination of repeat sizes; therefore, penetrance figures are based on results from the whole cohort. The curve provides insight into penetrance of HD with results in this size range. At age 55 years, penetrance was 40% (31-49%); at age 65 years, penetrance was 58% (46-48%); at age 75 years, penetrance was 74% (62-86%). These data will be valuable for counselling individuals with predictive test results in this size range.

Evidence of a genetic determinant responsible for a small proportion of neonates with non-random X-inactivation (NRXI). V. Bolduc¹, P. Chagnon¹, S. Provost¹, MP. Dube², C. Belisle¹, M. Gingras¹, L. Busque¹. 1) Research Center, Maisonneuve-Rosemont Hospital, University of Montreal, Montreal, Canada; 2) Montreal Heart Institute, University of Montreal, Montreal, Canada.

X-inactivation is a random process occurring in every female cell during embryogenesis. Females are thus mosaics of 2 cell populations, usually with an approximate 1:1 distribution of cells with active paternal X (Xp) and active maternal X (Xm). However, a significant number of females present a deviation from this theoretical ratio, termed non-random X inactivation (NRXI). In the murine model, the determination of the X chromosome to be inactivated is influenced by allelic variations at the *Xce* locus. In humans, despite the fact that some rare variants in the *XIST* gene have been associated with preferential inactivation, an *XCE*-like locus has not yet been characterized. To determine the genetic basis of NRXI in humans, and eventually identify its responsible elements, we first studied the transmission of X-inactivation patterns as a qualitative trait (using a criteria for NRXI >3:1) in mother-neonate pairs. Blood samples (fetal cord or peripheral) of 350 neonates and their mother were phenotyped using the HUMARA assay. The transmission of NRXI was not observed, as the incidence of NRXI found in neonates of mothers with or without NRXI is comparable (12.0% and 11.2%, respectively). Second, we performed linear regression analysis and correlation tests on the ratio of activation of the shared HUMARA allele of mother-neonate pairs. Because the HUMARA locus is in linkage with the X-inactivation center (<5 cM), such analysis may reveal the influence of the latter on NRXI. The results show a slight, but significant, correlation ($r=0.131$, $p=0.028$) of the X-inactivation ratios between mothers and their newborn for the transmitted allele. These preliminary results suggest that aside from stochastic events, NRXI may also be determined by a genetic component. That component may regulate selection of the X that becomes inactivated, or the cell survival following X-inactivation. Future work is needed to identify the gene(s) responsible.

Familial aggregation of a broadly defined phenotype of atrial fibrillation in Iceland. *D.O. Arnar¹, S.*

Thorvaldsson², G. Thorgeirsson¹, H. Hakonarson², K. Stefansson². 1) Landspítalinn University Hospital, Reykjavik, Iceland; 2) deCODE genetics Inc., Reykjavik, Iceland.

Atrial fibrillation (AF) is a common and serious disorder with known clustering in rare selected families. However, its heritability on a broad population basis has not been widely explored. We examined the heritability of AF in Iceland utilizing the Icelandic genealogy database and population-based data on patients with electrocardiographically-documented AF diagnoses. The study population consisted of 5,269 patients diagnosed since 1987 at the single university hospital in Iceland. Age-sex-matched controls were randomly selected from the genealogy database. Kinship coefficients (KC) were calculated before and after exclusion of relatives separated by 1-5 meiotic events and expressed as the genealogical index of familiarity (GIF) = average KC x 100,000. The average pairwise KC among patients with AF was 15.8 [95%CI of controls = 13.5,14.0]; this declined to 15.3 [13.3,13.8] after exclusion of relatives separated by 1 meiosis, 13.7 [12.3,12.8] after exclusion of 2 meioses, 12.7 [11.6,12.0] after exclusion of 3 meioses, and 11.2 [10.3,10.7] after exclusion of 4 meioses (all $p < 0.01$). Risk ratios among relative pairs declined incrementally, from 1.77 [95%CI, 1.69,1.94] in first degree relatives to 1.36, 1.18, 1.09, and 1.05 [1.03,1.07] in second through fifth degree relatives, respectively, consistent with the decrement among relative pairs in proportion of alleles shared identical by descent. In conclusion; AF shows strong evidence of heritability among unselected patients in Iceland, suggesting that there may be undiscovered genetic variants underlying AF on a broad population basis.

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Simulation of P-Values in MCMC-based Non-parametric Linkage Analysis. *G. Brock, D. Weeks, E. Feingold.* Dept Human Genetics, GSPH, Univ Pittsburgh, Pittsburgh, PA.

The significance level of a non-parametric linkage statistic is often found by simulation since the distribution of the test statistic is complex and unknown. Ideally, simulation occurs by assigning founder genotypes and then `dropping' genotypes through the pedigree. This situation mimics the actual pedigree data, where IBD sharing information is not known with certainty. However, this approach is usually computationally infeasible for larger pedigrees which require MCMC techniques to calculate the statistic, as an additional MCMC run is required to estimate the statistic for each gene-drop. One alternative is to drop inheritance vectors rather than genotypes, but this assumes that IBD sharing is known unambiguously and results in an overestimate of the variance and therefore a conservative significance level. In this work, we propose a novel method to estimate the significance level of the statistic. This is accomplished by estimating the Markov chain variability using the first gene drop, and then estimating the variability due to gene-dropping by running shorter MCMC chains on the additional simulated datasets. The two estimates are combined to form an overall estimate of the NPL statistic variability.

Higher heritabilities for volumetric density in weight-bearing versus non-weight-bearing bones: the Tobago

Family Study. X. Wang¹, CM. Kammerer¹, V. Wheeler³, AL. Patrick³, JA. Cauley², CH. Bunker², JM. Zmuda². 1) Dept Human Genetics, Univ Pittsburgh Sch Pub Hlth, Pittsburgh, PA; 2) Dept Epidemiology, Univ Pittsburgh Sch Pub Hlth, Pittsburgh, PA; 3) Tobago Health Studies Office, Trinidad & Tobago.

Cortical and trabecular bone density (BMD) and architecture influence the mechanical strength of the skeleton. However, genetic studies in humans have focused on areal measures of BMD, which reflect both cortical and trabecular bone. Little is known about the distinct and skeletal site specific genetic regulation of cortical and trabecular BMD in humans. Moreover, the genetic and environmental factors that affect bone strength phenotypes in populations of African ancestry, who have a very low risk of skeletal fragility, remain unclear. Using preliminary data on 120 women and 76 men (average age 42 yrs) from 7 multigenerational Afro-Caribbean families, we estimated heritability, and effects of age and sex, on cortical and trabecular volumetric BMD and cortical thickness for the distal and proximal tibia and non-dominant radius as measured by quantitative computed tomography. For both tibia and radius, age and sex effects accounted for a larger proportion of the total variation in cortical thickness (28 and 34%;), than in BMD (range from 9 to 25%). In addition, heritabilities of cortical BMD, trabecular BMD and cortical thickness of the tibia (0.570.16, 0.500.13, 0.380.13, respectively) were higher than those of the radius(0.130.11, 0.370.14, 0.280.11). Our preliminary analyses describe for the first time a major contribution of genes to interindividual variation in trabecular and cortical volumetric BMD among individuals of African descent. The results also indicate that factors such as age and sex have stronger effects on cortical thickness than on BMD. Additionally, genes appear to contribute to more of the interindividual variation in weight-bearing versus non-weight-bearing bones, indicating the presence of genotype by environment effects on bone phenotypes. Further studies of trabecular and cortical volumetric BMD should advance our understanding of the genetic and environmental determinants of bone strength.

Tetrahydrobiopterin responsiveness in phenylketonuria (PKU). *B. Burton, D. Hartung.* Genetics, Children's Memorial Hosp, Chicago, IL.

A significant fraction of patients with mild PKU respond to BH4 with a lowering of the blood phenylalanine (phe) level. Most investigators have found that very few patients with classical PKU exhibit a similar response. Recently, however, Matalon and Koch, et al (Genet Med 2004; 6:27-32) reported that 46% of classical PKU patients in their clinics responded to BH4. We tested 30 PKU patients (25 classical, 4 mild, 1 undefined) for BH4 responsiveness. Patients gave informed consent then were instructed to increase phe intake and/or decrease medical food intake for 3 days prior to taking a single dose of 6-BH4 20 mg/kg (maximum dose 1200 mg). Blood phe was measured at 0, 4, 8 and 24 hours. PKU mutation testing was performed for most subjects. BH4 responsiveness was defined as a decrease in blood phe level of at least 30%. 4 subjects were found to be BH4 responsive, 3 with mild PKU and one with an undefined phenotype. None of the 25 patients with classical PKU were BH4 responsive, although 1 subject had a 25% decline in blood phe level. The mean blood phe level at 0 time in the non-responsive subjects was 1121umol/L. The results for the BH4 responsive subjects are given below:

1	Mild	370, 200, 145, 152	L348V/R408Q
2	Mild	455, 291, 236, 339	F39del/A345S
3	Mild	394, 267, 152, 315	R408W/A345S
4	Undefined	800, 666, 297, 242	Pending

In our experience, few patients with classical PKU with high blood phe levels respond to a single high dose of BH4. Nonetheless there may be individual patients who will respond and it is possible that chronic treatment will result in a response that is not apparent after ingestion of a single dose. Further studies will be needed to address these questions.

Pediatric aspects of Fabry disease. *A. Raas-Rothschild¹, R. Backenroth², Y. Aurbach³, R. Weintraub-Shteyer⁴, A.J. Rein⁵, M. Zeigler¹, C. Thevenot⁶, G. Bach¹, D.P. Germain⁶.* 1) Human Genetics, Hadassah Hebrew University Medical Center, Jerusalem, Israel; 2) Department of Nephrology, Hebrew University Medical Center, Jerusalem, Israel; 3) Maccabi Health Services, Jerusalem, Israel; 4) Pediatric Day Care Hospital, Hebrew University Medical Center, Jerusalem, Israel; 5) Division of Pediatric Cardiology, Hebrew University Medical Center, Jerusalem, Israel; 6) Department of Genetics. Hopital Europeen Georges Pompidou. 75015 Paris, France.

Fabry disease (FD) is an X-linked lysosomal storage disorder due to the deficiency of the enzyme alpha-galactosidase with subsequent accumulation of globotriaosylceramide (Gb3) in the lysosomes of various tissues leading to clinical symptoms. The progressive nature of the disease and its time course explain why most disease features are reported in adults. Hemizygous males are invariably affected with the following clinical features: acroparesthesias, angiokeratomas, corneal and lenticular opacities, hypohydrosis and end-organ disease (kidney, heart and brain). However, findings in early childhood have been only recently reported and discussed. Here, we present our observations on 13 affected children and describe some less frequently recognized manifestations of FD, such as failure to thrive, short stature, gastro-intestinal involvement and priapism. We also present the outcome of enzyme replacement therapy with agalsidase beta in 5 children. Children were treated 18 months to 3 months with a good safety profile. We propose to evaluate treatment efficacy on pain assessment, school absenteeism, growth, abdominal pain, diarrhea and sweating. Our experience may contribute to the knowledge of FD in pediatrics and to the discussion of when to initiate ERT in children.

Enzyme Replacement Therapy with Hematopoietic Cell Transplant in Hurler syndrome: Multicenter initial experience. *S. Grewal¹, R. Wynn², J. Abdenur³, B. Burton⁴, M. Gharib², C. Haase⁵, D. Sillence⁶, G. Tiller⁷, P. Woodard⁸, N. Wulffraat⁹, G. Young³, C. Peters¹.* 1) Univ of Minnesota, Minneapolis, MN; 2) Manchester Childrens Hospital, UK; 3) CHOC, CA; 4) Childrens Memorial Hospital, Chicago, IL; 5) University of Jena, Germany; 6) Childrens Hospital Westmead, Australia; 7) Vanderbilt Medical Center, Nashville, TN; 8) SJCRH, Memphis, TN; 9) Childrens Hospital, Utrecht, NL.

Hematopoietic cell transplantation (HCT) can preserve intellectual function and improve survival in Hurler syndrome; however, it is associated with morbidity and mortality. Laronidase enzyme replacement therapy (ERT) can significantly reduce abnormal glycosaminoglycan (GAG) storage in many tissues, including the airways, leading to the hypothesis that it could mitigate HCT related complications. We report the initial multicenter experience with combined use of ERT and HCT in Hurler syndrome. Both pre- and post-HCT data were available on 10 patients. The median age at initiation of ERT was 13 months (range, 8-18 months), with a dose of 0.58 mg/kg/week. The median duration of pre-HCT ERT was 12 weeks (range, 6-24 weeks). All but 1 patient tested showed significant decrease in urinary GAG excretion during ERT. ERT infusion toxicity was mild. Development of antibodies to laronidase did not correlate with infusion reactions or responses in urinary GAG excretion. HCT preparative regimens varied by institution. Donor graft included cord blood, unrelated or sibling bone marrow. ERT was given for a median of 12 weeks (range, 4-20 weeks) after HCT. Following HCT, seven patients demonstrated complete donor engraftment; three patients suffered graft failure. Two patients required ventilator support; three developed acute GVHD. One patient died of multiple complications including pulmonary hemorrhage. Nine patients are surviving with followup of 1-6 months. We conclude that in children with Hurler syndrome, ERT with HCT is feasible and appears well tolerated. Development of antibodies against enzyme does not appear to correlate with infusion reactions or response to ERT. A systematic prospective study is needed to determine the effect of concomitant ERT on transplant outcomes.

Hematopoietic stem cell transplantation in a mouse model of gyrate atrophy: Does bone marrow transplantation correct hyperornithinemia? *B. Ross*¹, *M. Collector*², *S. Sharkis*², *D. Valle*³. 1) Dept Gynecology and Obstetrics, Johns Hopkins Univ, Baltimore, MD; 2) Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA; 3) Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.

Gyrate atrophy of the choroid and retina is an autosomal recessive disorder that results from the absence of ornithine delta-aminotransferase (OAT) and is characterized by hyperornithinemia and progressive retinal degeneration that culminates in blindness. In unaffected individuals OAT is expressed in the retina, liver, kidney, and small intestine. Although the pathophysiology of the retinal degeneration is not completely understood, studies have demonstrated that lower serum ornithine levels correlate with diminished retinal damage. A mouse model of this disorder has been previously reported. To determine if bone marrow transplantation could serve as a therapeutic option, female GA mice were transplanted with an enriched population of GFP labeled hematopoietic stem cells from healthy male mice and the percent of engraftment and serum ornithine levels were serially monitored for up to one year. The sub-population of HSCs used had been shown in a prior study to convert to hepatocytes when co-cultured with injured liver separated by a barrier. Following a standard protocol, the animals were transplanted with 1 million or 10 million cells. Despite consistently high levels of engraftment, greater than 70%, there was no drop in serum ornithine levels. We conclude that although high levels of engraftment with HSCs can be achieved and maintained without liver injury the degree of liver engraftment is insufficient, thus there is no correlating drop in serum ornithine levels.

Long-Term Outcome of Stem Cell Transplant in a Patient with Hunter Syndrome. *B.T. Tinkle¹, R.E. Harris², D.N. Franz³, L.A. Bailey¹, G.A. Grabowski¹.* 1) Divisions of Human Genetics; 2) Hematology-Oncology and; 3) Neurology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Hunter syndrome (mucopolysaccharidosis type II) is an X-linked lysosomal storage disorder resulting in the accumulation of glycosaminoglycans in multiple tissues due to the deficiency of iduronate-2-sulphatase. Patients exhibit clinical variability but present with coarse facial features, dysostosis multiplex, cardiomyopathy, hearing loss, and central nervous system involvement. Death commonly occurs in the first decade of life. Bone marrow transplantation (BMT) has been performed on more than 30 patients. The majority do not survive and those that do either are thought to have a milder phenotype or do poorly. We report a patient with severe neurobehavioral symptoms who received stem cell transplant (SCT) before the age of 2 years. The first graft failed but the second, 6 months later, has remained fully engrafted for over 4 years. Despite significant neurobehavioral problems, the patient continues to make developmental progress with intensive behavioral interventions. Bony changes are seen but are minimal as are the joint contractures. Brain MRI has shown continuing improvements in the myelination pattern and spectroscopy has returned to normal. The heart valves remained thickened, but the hypertrophy has improved overall. Liver and spleen volumes continue to decline despite a normal growth pattern suggesting continued clearance. His severe neurobehavioral patterns suggest a more severe Hunter phenotype yet, despite earlier cases of BMT, he has not only survived but does not show the deterioration as would be expected from the natural history of this disorder. The direct effects of the transplantation are hard to assess and it remains unknown whether his course is due to the phenotype of his private mutation, the transplant approach used, or by random chance. Thus, transplantation should still be considered, albeit cautiously, in children with Hunter syndrome under the age of 2 years.

Treatment of carpo-tarsal nephropathic osteolysis with zoledronic acid. *I. Kaitila, P. Arikoski, R. Karikoski, E. Marttinen.* Dept Clinical Genetics and Hospital for Children and Adolescents, Helsinki Univ Central Hosp, Helsinki, Finland.

The pathogenesis of the progressive carpo-tarsal osteolysis with nephropathy (OMIM# 166300) is unknown. No mode of treatment has been proposed. To treat the probably secondary central osteoporosis and, possibly, slow down or prevent the progression of the osteolytic process in a 15-year-old boy, we infused intravenously a potent bisphosphonate, zoledronic acid, every three months for one year. The proband was the second child of healthy unrelated parents. The osteolytic process was detected at the age of two years in studies for the cause of painful swelling in wrists, hands, ankles and feet. Proteinuria and cataracts were also observed. At the age of 12 years arterial hypertension developed, and enalapril treatment was started. Osteolytic process in the periphery of the extremities has been insidiously progressive without pain, swelling or other signs of inflammation after the initial phase: the carpal and tarsal bones, and phalanges have almost completely disappeared. Proteinuria has persisted, and renal biopsy showed hyalinisation of glomeruli, thickening of mesangial matrix, and interstitial fibrosis. The renal function tests have remained normal. Plasma Ca, Pi, ALP, and PTH have constantly been within the normal range. The skeletal radiographs suggested central osteoporosis, and BMD at the LI-IV was 0.683 g/cm² (Z-score -1.60), and femoral collum 0.715 g/cm² (Z-score -1.2) at the age of 13 years. The first infusion of zoledronic acid (1 mg in 50 cc of physiologic saline over 15 min) resulted in the expected acute phase reaction (fever, skeletal pain), whereas the following three infusions had no adverse effects. Except for mild hypocalcaemia the laboratory tests remained within the normal range. In particular, the renal function tests did not reveal any deterioration. The skeletal radiographs did not reveal any objective effect of the medication. However, BMD at the LI-IV increased to 0.993 g/cm² (Z-score 1.0), and femoral collum to 0.840 g/cm² (Z-score - 1.1). The efficacy of bisphosphonates as treatment for osteolytic disorders remains to be evaluated.

Effect of L-Arginine on the Neurotransmission and the Mitochondrial Respiration of Mice Brains Synaptosome.

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Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS) is a maternally inherited multi-system mitochondrial disorder characterized by stroke-like episodes before 20 years of age. L-arginine (Arg), a potent vasodilator via endothelial function through nitric oxide production (NO), has been used for therapeutic trials in MELAS patients during the acute phase of stroke-like episodes. We found that Arg therapy quickly decreased severity of stroke-like symptoms in MELAS, enhanced dynamics of the microcirculation, and also reduced tissue injury from ischemia. In this study, we elucidate the biochemical effects of Arg on nerve endings. Endbrains from wild-type mice (ICR) were used throughout this study. Crude synaptosome was analyzed for their concentration of GABA and Glutamate (Glu) after preparations of several fractions. We analyzed the contents of releasing amino acids evoked by high potassium condition and uptake of them in three separated fractions (cytosol, vesicles and intact mitochondria). The oxygen consumption was measured by oxygen electrode. The entire uptakes of GABA and Glu were inhibited by rotenone (30 nmole/mg protein) with dose-dependent manner and showed a plateau at about 70% of total uptake. Arg inhibited the uptake of Glu logarithmically, however showed no change in uptake of GABA. The contents of GABA and Glu in synaptosome were decreased in the presence of Arg by inducing their release. The up regulation of GABA release without rotenone was occurred uniquely by Arg. Arg enhanced the respiration of state II by succinate on synaptosomal respiration, although the respiration of synaptosomal mitochondria fraction and the respiratory chains enzyme activities were almost unaffected by Arg. In conclusion, these results suggest that L-arginine may modulate the neuroexcitability on nerve endings uniquely and alleviate stroke-like episodes in MELAS.

Human foamy virus based gene transfer vectors the treatment of metachromatic leukodystrophy. *T. Rupa¹, J. Liu¹, C. Regan¹, E. Dodds², G. Patton², M. McClure².* 1) Biochemical Genetics Lab, Child & Parent Resource Inst, University of Western Ontario, London, ON, Canada; 2) Imperial College, London, UK.

The human foamy virus (HFV) is a Spumavirus in the genera of Retroviridae. HFV has a wide tissue tropism and host range, large genome size and is capable of integration into both dividing and non-dividing cells. Most importantly it is not pathogenic. Metachromatic leukodystrophy (MLD) is an inherited lysosomal storage disorder caused by a defect in the arylsulfatase A (ASA) gene. ASA catalyzes the first step in the catabolism of the myelin glycolipid sulfatide. There is no cure for MLD. HFV derived vectors expressing galactosidase (BGAL), green fluorescent protein (GFP) and ASA-GFP have been produced by triple transfection of 293T cells. These vectors have been used both in vitro and in vivo to study the expression of the transgenes in cultured murine oligodendrocyte N20.1 cells, cultured human fibroblasts from controls and patients with metachromatic leukodystrophy and in a murine ASA knockout model of MLD (ASA KO). HFV-LacZ vector was used to transfect the murine oligodendrocyte N20.1 cell line and 40-80% cells expressed BGAL after 72 hours. Six clones of permanently expressing BGAL have been used to locate the sites of genomic integration. In human MLD fibroblasts transfected with HFV-ASA-GFP, GFP was expressed in 5-10% of MLD fibroblasts. The results of ASA assay in a population of transfected human fibroblasts enriched in GFP positive cells demonstrated that the overexpressed ASA could efficiently hydrolyze both bodipy sulfatide and the artificial substrate (p-nitrocatechol sulfate). ASA overexpressed in the transfected human cultured fibroblasts was taken up by both cultured fibroblasts from human MLD patients and by primary neural cell cultures of the ASA KO mouse and able to correct the ASA deficiency. HFV-lacZ, HFV-GFP and HFV-ASA-GFP gene transfer vectors were injected into the brains of ASA KO mouse. Reporter genes LacZ or GFP were expressed in cells around the injection site for at least three months without a decline in reporter signal intensity. These data indicate that HFV vectors may be effective as gene transfer vectors in the treatment of MLD.

Neural Progenitor Cells from an Adult Patient with Fragile X Syndrome. *P. Schwartz*^{1,2,3}, *F. Tassone*⁴, *C.M. Greco*⁵, *H.E. Nethercott*¹, *B. Ziaieian*², *R.J. Hagerman*⁶, *P.J. Hagerman*⁴. 1) National Human Neural Stem Cell Resource; 2) Stem Cell Research, Children's Hospital of Orange County Research Institute, Orange, CA; 3) Developmental Biology Center, School of Biological Sciences, University of California, Irvine, Irvine, CA; 4) Departments of Biochemistry and Molecular Medicine, Davis, CA; 5) Department of Pathology, Davis, CA; 6) M.I.N.D. Institute, University of California, Davis, School of Medicine, Davis, CA.

Fragile X syndrome, the leading heritable form of mental impairment, is a trinucleotide repeat disorder that is caused by methylation-coupled gene silencing for CGG repeat expansions greater than ~200. In an effort to develop a human cell-appropriate model to study this disorder, neural cells were successfully harvested and grown from post-mortem brain tissue of a 25-year-old adult male with fragile X syndrome. The cultured cells display many of the characteristics of neural progenitor cells as well as the biochemical hallmarks of fragile X syndrome. These neural progenitor cells do not express any FMR1 mRNA or FMRP. We further demonstrate initial efforts to differentiate these progenitor cells into both neuronal and astrocytic lineages. The successful production of neural cells from an individual with fragile X syndrome opens a new avenue for the scientific study of the molecular basis of this disorder, as well as an approach for studying the efficacy of new therapeutic agents.

SMN mRNA and protein: surrogate biological markers of drug-response in Spinal Muscular Atrophy (SMA)

clinical trials. L.R. Simard¹, M. Hang², S. Morissette¹, M.-C. Bélanger¹, M. Wride², J. Johnson², K.J. Swoboda². 1) Centre de recherche de l'Hôpital Sainte-Justine, Université de Montréal, Montréal, PQ, Canada; 2) University of Utah, Salt Lake City, Utah, USA.

SMN2, a duplicate copy of the *SMN1* gene, is the only known modifier gene of SMA, a devastating childhood neurodegenerative disease. The timing and extent of motoneuron cell death is most likely a dosage effect of residual SMN protein. Anticipating upcoming clinical trials using compounds that either alter SMN splicing or up-regulate SMN expression, we developed and validated sensitive methods amenable to multi-center drug trials to measure SMN mRNA and protein in specimens collected from SMA patients. Blood samples were collected from 40 patients and 23 carriers for about 4 visits per individual to assess variability in the same individual over time. Total mRNA was prepared using the PAXgene Blood RNA system and total protein from mononuclear cells, platelets, buccal cells or skin fibroblasts. Full-length (7in) and exon 7-lacking (7out) transcripts were quantified by real time RT-PCR using fluorescent probes. Normalized results indicated that SMN expression remained relatively stable over time and was quantitatively different between patients, carriers and controls. Given extensive inter-individual variability, there was no correlation between the 7in:7out ratio and *SMN2* copy number. This assay can reliably measure and distinguish the mechanism of drug-response. Analysis of SMN protein in buccal cells was aborted due to extensive non-specific cross-reactive material. While skin fibroblast yielded clean results, there was no advantage compared to blood. Thus, protein was examined by Western blot analysis using platelets and mononuclear cells prepared from whole blood. Results for SMN protein were similar to SMN mRNA in that SMA patient samples showed the lowest amount of SMN protein in both platelets and mononuclear cells whereas controls and carriers showed the highest and intermediate amounts, respectively. Our data supports the reliability and potential usefulness of these assays in assessing SMN mRNA and protein as surrogate markers in therapeutic trials. *Funded by Families of SMA.*

Systemic gene transfer of AAV6-IGF-1 slows disease progression in a mouse model of ALS. *P. Weydt¹, S. Abmayr², J. Allen², P. Calses¹, L. Meuse², A.R. La Spada^{1,2,3}, J. Chamberlain².* 1) Department of Laboratory Medicine, University of Washington, Seattle, WA; 2) Department of Neurology, University of Washington, Seattle, WA; 3) Division of Medical Genetics, Department of Medicine, University of Washington, Seattle, WA.

Amyotrophic lateral sclerosis (ALS) is a paralyzing, invariably fatal adult-onset neurodegenerative disorder, which is defined by a progressive and selective loss of the motor neurons of the spinal cord and the brain. ALS affects 8 in 100,000 people per year and there is currently no cure. Recent studies have demonstrated that intramuscular injections with viral vectors expressing neurotrophic factors such as insulin-like growth factor 1 (IGF-1) or vascular endothelial growth factor (VEGF) can substantially extend survival in the SOD1-transgenic mouse model of ALS. The therapeutic effect is thought to involve retrograde axonal transport of a small fraction of the injected vector from the intramuscular injection site to the motoneuron cell bodies in the spinal cord. Intravenous delivery of recombinant adeno-associated virus pseudotyped with serotype 6 capsids (rAAV6) is an efficient way of targeting the entire muscle mass of the body without the need and risks of multiple intramuscular injections (Gregorevic et al, Nat Med, 2004, in press). Consequentially this method has the potential to target all lower motoneurons. We explored how systemic injection of the rAAV6 vector affects disease progression in the G93A SOD-1 transgenic mouse model of ALS. These mice uniformly develop a progressive motor paralysis around 90 days of age, which is typically fatal at 120-130 days of age. We report that a single IV injection of 10^{12} viral genomes of rAAV6/CMV-mIGF1 per animal at 60 days of age slows disease progression significantly and prolongs survival by about 19 days (22%). This result demonstrates that AAV6 is a safe and useful method to deliver therapeutic doses of IGF1 to the motor system.

Correction of Hurler syndrome with a single IV administration of lentiviral vector to newborn mice. C.B.

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Mucopolysaccharidosis type I is a lysosomal disorder due to deficiency of -L-iduronidase (IDUA). As illustrated here, the knockout mouse is a valid model of human disease, exhibiting metabolic defects, dysmorphology and learning defects analogous to affected children. A lentiviral vector expressing IDUA enzyme was administered intravenously to newborn mice with Hurler syndrome. After this single injection, plasma levels of IDUA enzyme were increased in all treated mice throughout the 100-day study period (0.1- to 60-fold of normal heterozygote level), and reached levels 2- to 60-fold above normal in 7 of 10 mice. In 6 of 10, hepatic IDUA enzyme levels were 4- to 17-fold above normal. Significant amounts of IDUA enzyme were found in spleen (2-20%; of normal heterozygote), heart (2-11%;), brain (1.5-7%;) and peripheral blood leukocytes (1.7-10%;). High levels of transgene were detected in liver (1-115%;, ie, 1 - 115 IDUA gene copies per 100 genome equivalents), heart (0.05-23%;) and spleen (0.06 - 4.1%;). Metabolic correction was indicated by the complete absence of pathology in liver and kidney in most mice, and by absence of facial dysmorphology in all mice. In mice with the highest circulating enzyme levels, pathologic GM2- and GM3-ganglioside were markedly reduced in the brain, and this effect was inversely related to the level of enzyme. Notably, a learning abnormality was identified, but was absent in treated mice. Transplantation of marrow into another generation of knockout mice demonstrated persistence of IDUA transgene thus demonstrating gene transfer into hematopoietic stem cells, a potential conduit for IDUA enzyme across the blood-brain barrier. These observations are the first demonstration of metabolic and phenotypic correction of a disorder by in vivo gene therapy after neonatal treatment with a lentiviral vector.

RNAi mediated gene therapy for dominantly inherited vascular Ehlers-Danlos syndrome (EDSIV). A. Watanabe^{1, 2}, K. Ter³, T. Wada⁴, Y. Fukushima⁴, T. Shimada^{1, 2}. 1) Dept Biochemistry & Molec Biol, Nippon Medical Sch, Tokyo, Japan; 2) Div clinical Genetics, Nippon Medical Sch Main Hosp, Tokyo, Japan; 3) UPBSB, Dept Biophysics & Biochem, School of Sci, Univ of Tokyo, Tokyo, Japan; 4) Div Clinical & Molec Genet, Shinshu Univ Hosp, Matsumoto, Japan.

Vascular Ehlers-Danlos syndrome (vEDS, also known as EDSIV), is a life-threatening dominantly inherited disorder caused by mutations in the gene for type III procollagen (*COL3A1*). Affected individuals are at risk for arterial, bowel and uterine rupture. There is no effective treatment to prevent the symptom associated with vEDS. Although gene therapy is an important option for treatment of genetic disorders, the current strategy based on addition of the defective gene is not applicable for dominant diseases such as vEDS. RNA interference (RNAi) may hold therapeutic promise for silencing dominantly acting genes. Approximately one third of the known mutations of vEDS occur at 5 donor splice sites. Therefore, if aberrantly spliced mRNA could be selectively destroyed by RNAi, it might be possible to reduce the mutant *COL3A1* level and increase the relative concentration of normal triple helix. In this work, we examined the feasibility of the RNAi mediated gene therapy for vEDS. The target is a substitution of A for G+ 1 at the 5 site of intron 20 of the *COL3A1* gene (G+1 IVS20) which generates mutant mRNA molecules containing either 24 nucleotides from intron 20 or the total intron 20 sequence. We synthesized several small interfering RNA (siRNA) molecules targeting the extra intron 20 sequence and introduced into patients fibroblasts with the G+1 IVS20 mutation. Expression of normal and mutant *COL3A1* mRNA were quantitatively measured after RT-PCR. The results indicated that mutant mRNA levels could be selectively decreased up to 70 % by transient transfection of one of the siRNA molecules. These studies strongly encourage the RNAi mediated allele specific gene silencing for dominant disease gene. A major shortcoming of this approach is the transient nature of the effect due to the intracellular degradation of siRNA. The use of integrating viral vector for stable expression of siRNA may be feasible for treatment of vEDS.

Congenital Leptin Deficiency due to Homozygosity for the 133G Mutation: Report of a Further Case and Evaluation of Response to Four Years of Leptin Therapy. *W. Gibson¹, I.S. Farooqi², M. Moreau³, A.M. DePaoli⁴, E. Lawrence⁴, S. O'Rahilly², R.A. Trussell³.* 1) Department of Medical Genetics, B.C. Children's Hospital, Vancouver, British Columbia, Canada; 2) University Department of Clinical Biochemistry, Cambridge Institute for Medical Research, Addenbrooke's Hospital, Cambridge, UK; 3) Department of Paediatric Endocrinology, Alberta Children's Hospital, 1820 Richmond Rd. SW, Calgary, Alberta, Canada; 4) Amgen Inc., One Amgen Center Drive, Thousand Oaks, CA, USA.

Congenital leptin deficiency is a rare, but treatable, cause of severe early-onset obesity. To date, two UK families of Pakistani origin carrying a frameshift/premature stop mutation c.398delG (133G) and one Turkish family carrying a missense mutation c.313CT (Arg105Trp) have been described. Affected subjects are homozygotes and manifest severe obesity and hyperphagia accompanied by metabolic, neuroendocrine and immune dysfunction. The effects of recombinant leptin therapy have been reported in three children with the 133G mutation, and in all cases this has led to a dramatic resolution of clinical and biochemical abnormalities. We now report a Canadian child, of Pakistani origin but unrelated to the previously reported subjects, presenting with severe hyperphagia and obesity, who was found to be homozygous for the 133G mutation. In this child, four years of therapy with subcutaneous injections of recombinant leptin provided further evidence for the sustained beneficial effects of leptin replacement on fat mass, hyperinsulinaemia and hyperlipidaemia. In addition, leptin administration corrected abnormal thyroid biochemistry and allowed the withdrawal of thyroxine treatment, providing further support for the role of leptin in the regulation of the human hypothalamic-pituitary-thyroid axis.

Clinical presentation and management of Hemoglobin Buenos Aires: conundrums. *R.M. Roberts¹, L.M.S. Resar², T. Hays³*. 1) Genetics and Prenatal Diag Ctr, Sarasota, FL; 2) Johns Hopkins University School of Medicine, Baltimore, MD; 3) University of Colorado Children's Hospital, Denver, CO.

Few case reports of Hb Buenos Aires (also known as Hb Bryn Mawr) exist. The clinical presentation varies, and only a few (possibly two) families have been tested to confirm new mutations, heterozygotes in the affected. Hb Buenos Aires is unstable because of abnormal avidity for oxygen, and the patient is at risk of significant hemolytic anemia and aplastic crisis with Parvovirus B19. Our patient presented with episodic undiagnosed splenomegaly associated with URI and streptococcal pharyngitis at ages 4 and 5, the latter with an elevated reticulocyte count as the only hematological abnormality noted. At age 6, lab workup for another strep pharyngitis associated with splenomegaly revealed evidence of chronic stable hemolytic anemia, and the diagnosis of Hb Buenos Aires was made at the Johns Hopkins DNA Diagnostic Lab after Hematologic referral. The patient moved to Denver, where at age 7 developed aplastic crisis following Parvovirus B19 infection, requiring transfusion after Hb fell to 5.0 (the first Japanese case presented with aplastic crisis in a 9-year old girl in 1992). Hb has since been in the range of 10 to 12, but fell to 7 following a minor strep infection characterized by jaundice, splenomegaly and abdominal pain. A 2nd Japanese case was reported in 2002 of compensated hemolytic anemia with jaundice, reticulocytosis, and Hb 12-13 in a 12 y.o. boy while stable, with episodes of hemolytic crisis and aplastic crisis. Some evidence suggests that tissue hypoxia does not occur because of the increased avidity to O₂, possibly contradictory to suggested presurgical management with erythrocytapheresis advocated in Hb Bryn Mawr by Larson et al in 1997. Further unresolved questions: do these patients merit splenectomy or is the surgical and post-splenectomy risk of death by bacterial sepsis too high? Are some heterozygotes asymptomatic? Is the prevalence significantly underestimated? Does splenectomy significantly decrease the risk of hemolytic crisis?

Response to Very Early Copper Treatment in Classical Menkes Disease. *S.G. Kaler, P.C. Liu, J. Tang, K.E. Lem.*
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Menkes disease (MD) is a X-linked recessive disorder of copper transport caused by mutations in a copper-transporting ATPase (ATP7A). From July 1995 to June 2004, we conducted a clinical trial of early copper therapy in 16 previously unreported infants with MD, using a single formulation of copper histidine (CuHis, IND #34,166). Mean age on study entry was 11.8 d (range: 5-30 d). No infant showed neurological symptoms upon entry; diagnosis was by plasma or CSF neurochemical analysis, with molecular confirmation. CuHis dose was 250 mcg by subcutaneous injection twice daily until age 12 mos, and then once daily, to 3 yrs. Primary outcome parameters included clinical neurodevelopment, brain MRI, EEG, serum and CSF copper, plasma and CSF catecholamine ratios, mutation at the ATP7A locus, and confocal microscopic imaging of cultured patient fibroblasts. Four patients with detectable ATP7A protein on confocal analysis achieved independent ambulation at or before 15 mos of age and are neurologically normal at ages ranging from 3 to 9 yrs. Twelve patients with less successful neurodevelopmental outcomes, including four who died by age 3 yrs despite very early intervention, showed little or no ATP7A on confocal imaging. The latter group included patients with large deletions and frameshift mutations. Post-mortem analysis of brain copper was available for one such patient and indicated failure of copper histidine to cross the blood-brain barrier into the central nervous system, despite restoration of normal blood copper levels. In responder patients, CSF catechol ratios were four-fold lower (indicating less profound deficiency of dopamine-beta-hydroxylase) compared to non-responders. We conclude that baseline determination of CSF catechol ratios, mutation type, and presence of residual ATP7A by confocal microscopic analysis of cultured cells predict a subset of patients with classical MD in whom early copper histidine therapy will normalize neurodevelopmental outcome. For other patients, in whom residual ATP7A activity is not evident, alternative approaches to breach the blood-brain barrier (e.g., intrathecal copper histidine administration) appear warranted.

Fetal Cells Appear to Participate in the Maternal Response to Wound Healing. *P.K. Khosrotehrani*^{1,2}, *S. Guegan*^{1,2}, *K.L. Johnson*², *G. Sahagian*³, *K. Tao*³, *D.W. Bianchi*². 1) Dept Dermatology, Hopital Tenon, Paris, France; 2) Dept Pediatrics, Division of genetics, Tufts-New England Medical Center, Boston, MA, USA; 3) Dept of Physiology, Tufts University School of Medicine, Boston, MA, USA.

Background: In humans, fetal cells with stem-cell capabilities enter the maternal circulation and persist for decades. We recently reported that fetal cells could adopt the maternal injured tissue phenotype and that they express non-hematopoietic markers (JAMA 2004; in press). Our objective was to determine whether fetal cells participate in maternal wound healing in a mouse model. **Methods:** We mated female wild-type FVB mice to males transgenic for the luciferase gene under the control of the constitutively expressed CMV promoter (CMV:Luc) (n=2) or the transcriptionally-regulated angiogenic factor VEGFR2 promoter (VEGFR2:Luc) (n=11) or to wild-type males as controls (n=2). At various times during and after pregnancy we performed a 6mm punch skin biopsy. The presence of the luciferase-expressing fetal cells in the maternal wound was assessed using in vivo whole animal imaging. **Results:** In the absence of injury, no luciferase signal could be visualized in any area outside the utero-placental signal corresponding to the transgenic fetuses. Three days after inducing injury in wild-type female mice mated with CMV:Luc males, luciferase activity, associated with the presence of transgenic fetal cells, could be visualized in the wound area but not in the surrounding healthy tissues. Similarly, a specific signal was observed in the wound of female mice mated with VEGFR2:Luc males. Luciferase activity was seen in both pregnant and post-partum mice. No signal was observed in control female mice mated to wild-type males that produced no transgenic fetuses. **Conclusions:** We demonstrate that fetal cells transferred during pregnancy specifically home to the maternal skin wound and express VEGFR2, a marker for wound healing and angiogenesis. Fetal cells appear to participate in the maternal wound healing process. Studies of adult stem cells need to consider the possibility that some of the cells with observed plasticity derive from the fetus or placenta.

Clinical response to enzyme replacement therapy in pediatric patients with type 1 Gaucher Disease. H.C.

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50% of type 1 Gaucher disease (GD1) patients are diagnosed before 18y. No long-term assessment of clinical response to enzyme therapy (ERT) in pediatric patients has been reported. We report the response to ERT in 433 symptomatic GD1 patients under 18y in the Gaucher Registry, an international registry established in 1991. Minimum average ERT dose was 30u/kg/infusion q2wks and none had splenectomies.

DISEASE MANIFESTATION	BASELINE PREVALENCE	PREVALENCE AFTER ERT
Anemia	42%	18% at 36mo ERT
Low platelets(<120K/cu mm)	63%	16% at 36mo ERT
Low platelets (<60K/cu mm)	9%	0% at 48mo ERT
Splenomegaly (>15MN)	69%	10% at 36mo ERT
Hepatomegaly (>1.25MN)	92%	36% at 48mo ERT
Growth, >2SD below mean ht	25%	15% at 48mo ERT
Growth, below mean ht	80%	65% at 48mo ERT

Longitudinal curves for each parameter over 10y ERT show sustained benefits. Clinical response was greatest in patients with severest pathology. These data allow the first prediction of ERT responses in pediatric GD1.

Overall treatment effects of agalsidase alfa in Fabry disease: results from FOS - the Fabry Outcome Survey. *M. Beck*¹, *A. Mehta*², *G. Sunder-Plassmann*³, *U. Widmer*⁴. 1) Department of Paediatrics, University of Mainz, Mainz, Germany; 2) Department of Haematology, Royal Free Hospital, London, UK; 3) Department of Medicine III, Medical University, Vienna, Austria; 4) Department of Medicine, University of Zurich, Zurich, Switzerland.

Fabry disease is a rare X-linked disorder caused by deficient activity of the lysosomal enzyme -galactosidase A. Progressive accumulation of the substrate globotriaosylceramide in cells throughout the body leads to major organ failure and premature death. The Fabry Outcome Survey - FOS - is a European outcomes database which was established to collect data on the natural history of this little-known disease and to monitor the long-term efficacy and safety of enzyme replacement therapy (ERT) with agalsidase alfa. We report here an analysis of the FOS database on the effects of ERT on renal function, heart size, pain and quality of life in a cohort of 314 patients (203 males, 111 females) given agalsidase alfa (0.2 mg/kg infused over 40 minutes every 2 weeks) for up to 2 years. Treatment with agalsidase alfa stabilized renal function (as assessed by estimated glomerular filtration rate) in patients with a mild or moderate deterioration in renal function at baseline, reduced left ventricular size (as assessed by echocardiography) in patients who had an enlarged heart at baseline, and improved pain scores and quality of life (as assessed by questionnaire responses to the Brief Pain Inventory and the European Quality of Life Questionnaire EQ-5D). These improvements were similar in hemizygous men and heterozygous women with Fabry disease. The incidence of infusion reactions was approximately 0.7%; these were mild and responded to slowing or temporary cessation of the infusion or to premedication where necessary. There were no serious adverse events related to treatment. In conclusion, ERT with agalsidase alfa has significant clinical benefits in patients with Fabry disease, and appears to be able to alter the natural history of the disorder.

Monthly intrathecal enzyme replacement therapy improves lysosomal storage in the brain and spinal cord in canine MPS I. *P. Dickson¹, M. McEntee², C. Vogler³, S. Le¹, B. Levy³, P. Belichenko⁴, W. Mobley⁴, S. Hanson⁵, M. Peinovich¹, M. Passage¹, E. Kakkis^{1,6}.* 1) Harbor-UCLA Medical Ctr., Torrance, CA; 2) Dept. of Pathol., Coll. of Vet. Med., U. of Tenn., Knoxville, TN; 3) Dept. of Pathol., St. Louis U., MO; 4) Dept. of Neurol., Stanford Univ., Stanford, CA; 5) Veterinary Neurol. Ctr., Tustin, CA; 6) BioMarin Pharmaceutical Inc., Novato, CA.

Intrathecal recombinant human -L-iduronidase (IT rhIDU) given weekly in canine mucopolysaccharidosis I (MPS I) reduces CNS (central nervous system) glycosaminoglycan (GAG) storage. Monthly IT rhIDU was tested to determine whether the frequency of IT injections could be reduced while maintaining efficacy. Three MPS I dogs received ~1 mg of IT rhIDU in 4 monthly doses along with weekly intravenous (IV) rhIDU. Iduronidase levels reached 23-fold normal in brain, 7-fold in spinal cord, and 423-fold in meninges (vs. 23, 13, and 300-fold in 4 dogs treated with only IT rhIDU in 4 weekly doses). Brain GAG was 4.07 ± 0.70 g/mg tissue weight, vs. 8.26 ± 1.23 in 4 untreated MPS I dogs (p=0.003). Brain GAG with monthly IT and weekly IV rhIDU was not significantly different from weekly IT (4.47 ± 0.69, p=0.48), and approached levels in untreated normal dogs (5.40 ± 1.82, p=0.22). GAG levels in spinal cord were 3.93 ± 0.70 g/mg dry weight in monthly treated MPS I dogs, versus 5.04 ± 0.93 in untreated MPS I dogs and 3.02 ± 0.71 in untreated, normal dogs (p=NS). One dog had neurologic signs, including depressed mental status, ataxic gait, absent gag reflex, and head tilt, which improved with monthly rhIDU treatment. Two of three monthly-treated dogs developed a mild antibody response in blood and CSF. One dog had been made tolerant to rhIDU using a new method and had virtually no immune response in blood or CSF, and only a minimal response pathologically. Monthly IT with weekly IV rhIDU may be as effective as weekly IT alone in correcting the enzyme deficiency and lysosomal storage (measured by GAG levels) in the CNS in canine MPS I.

Marked improvement of leukodystrophy in a child with Hurler-Scheie Disease treated by enzyme substitution therapy. *B. Heron-Longe*¹, *C. Baumann*³, *S. Magnier*⁴, *P. Wicart*², *A. Verloes*³, *G. Ponsot*¹. 1) Pediatric Neurology Department, Hopital St Vincent de Paul, Paris, France; 2) Paediatric Surgery Department, Hopital St Vincent de Paul, Paris; 3) Clinical Genetics Department, Hopital Robert Debre, Paris; 4) Pediatric Cardiology Department, Hopital Robert Debre, Paris.

Type I mucopolysaccharidosis (MPS I) is a lysosome storage disease due to a deficiency in α -L-iduronidase. It gives rise to the onset and gradual worsening of joint and bone, cardiac, respiratory, digestive, sensorial and neurological complications. A distinction can be made between the most severe forms, or Hurler Disease with involvement of the central nervous system (CNS) and premature death, attenuated forms without dysmorphism and CNS damage or Scheie Disease, and intermediate forms, or Hurler-Scheie Disease. The only therapy available until recently was bone marrow transplant, on condition that a compatible donor could be found and that CNS damage was absent or moderate; even so, there was a high risk of fatality. Laronidase, a recombinant form of human α -L-iduronidase (Aldurazyme) is an enzyme replacement therapy for MPS 1. Studies of its efficacy concerned the non-neurological symptoms of the disease in patients aged between 5 and 65 years. The effects of this treatment on the central neurological symptoms of MPS 1 have not yet been studied and it seems to be no diffusion of the enzyme through the brain blood barrier. We report herewith on the case of a young, 7-year-old boy suffering from an intermediate form of MPS I, with retarded learning skills and leukodystrophy detected on a cerebral MRI scan. Enzyme replacement therapy was initiated and current experience of the outcome under treatment is now 18 months. No adverse effects have been observed. There was an improvement in the patient's general status and joint function, and a disappearance of visceromegaly. A clear regression of leukodystrophy was also observed. The latter observation is suggestive of a beneficial intracerebral effect of intravenous Aldurazyme and may also be suggestive of its passage into the brain.

The role of enzyme replacement therapy in the management of Hurler's syndrome. *J. Hersh¹, S. Soni², M. Hente², S. Bertolone², N. Breslin², C. Whitley³, A. Cheerva².* 1) Dept Pediatrics/WCEC, Univ Louisville, Louisville, KY; 2) Division of Pediatric Hematology/Oncology and BMT, Univ Louisville, Louisville, KY; 3) Gene Therapy Program, Univ Minnesota Medical School, Minneapolis, MN.

Hurler's syndrome (mucopolysaccharidosis type I) is a progressive multisystem disorder that results from deficient (alpha)-1-iduronidase. Major manifestations include mental retardation, macrocephaly, corneal clouding, coarse facies, hearing loss, joint stiffness, hepatosplenomegaly, obstructive airway disease, valvular heart disease and short stature. The diagnosis usually is established early in life based on clinical manifestations. Hematopoietic stem-cell transplantation is the treatment of choice in affected children under age 2 years, when there is minimal or no central nervous system disease. Enzyme replacement therapy (ERT) with recombinant (alpha)-1-iduronidase (laronidase) has been recommended for milder or attenuated forms of mucopolysaccharidosis type I. We present a Hispanic male diagnosed with Hurler's syndrome at age 7 months. Although donor cells were available, he was unable to undergo transplantation because of severe respiratory disease requiring tracheostomy and frequent hospitalizations. Following 12 weeks of ERT, there was dramatic improvement clinically enabling successful transplantation. An additional 10 weeks of ERT was given beginning 4 weeks post-transplantation until the transplanted marrow was well engrafted. Only minimal antibody titers to enzyme have been detected 3 months after discontinuing ERT, with continued rise of (alpha)-1-iduronidase levels and decrease in urinary glycosaminoglycans. Our patient's response to ERT both prior to and after hematopoietic stem-cell transplantation, lends support to considering its use 6-12 weeks before, and up to 3 months following the transplantation procedure, as a valuable adjunct in the medical management of Hurler's syndrome, in anticipation of endogenous enzyme production by donor cells.

Intrathecal Delivery of Iduronate 2-Sulfatase for MPS II to the Canine CNS. *J.C. Lamsa, A. Garcia, K. Loveday, J. Pan.* Research, TKT, Cambridge, MA.

Iduronate 2-sulfatase (IdS) was delivered to normal beagle dogs intrathecally (IT) via the cisterna magna. Bolus 1 mL IT injections of either 2 (N = 2) or 10 mg (N = 2) IdS were administered. Intravenously (IV) injected (2 mg, N = 1; 10 mg, N = 1) and untreated (N = 1) animals served as controls. Serum levels of IdS measured by ELISA remained at control levels until at least 5 hours post IT injection. The half-life of IdS in CSF was estimated to be 2 to 3 hours. Brain and liver samples were collected either 4 or 24 hours after injection. No IdS was found in liver 4 hours after IT injection. Brain tissue levels of IdS as measured by ELISA were < 2 ng/mg protein in untreated or IV injected animals. In IT treated animals (2 mg) IdS levels were 2 to 5 fold higher than IV controls throughout the brain. At the 10 mg dose, IdS levels in brain were elevated as much as 20X compared to IV controls. Fluorescent immunohistochemistry for IdS revealed that the meninges and perivascular cells were the major sites of uptake at both dose levels. At 4 hrs post IT injection, neurons and glial cells of the cerebral cortex were negative for IdS. Within 24 hrs after IT injection of 10 mg IdS neuronal and glial uptake was evident in approximately 25% of cells in the cerebral cortex compared to approximately 10% at the 2 mg dose. Two additional animals received 3 mg IdS by IT injection weekly for 4 weeks. IdS uptake was similar to a single 10 mg IT injection in terms of number of positive neurons, but the staining intensity was equivalent to the higher dosage in 1 dog, and higher in the other. These latter data indicate that IdS accumulated within neurons after repeated injection, and that the tissue half-life of IdS may be several days. We conclude that IT injection may prove valuable in treating MPS disease with CNS complications.

Low-Dose ERT for Gaucher Disease in So Paulo State (Brazil). *A.M. Martins¹, E. Sobreira², T.C. Bortolheiro³, G. Porta⁴.* 1) Centro de Referencia em Erros Inatos do Metabolismo, Universidade Federal de So Paulo, So Paulo, SP, Brazil; 2) Departamento de Pediatria e Puericultura, FCM Santa Casa de So Paulo, So Paulo, SP, Brazil; 3) Departamento de Clinica Mdica, Servio de Hematologia, FCM Santa Casa de So Paulo, So Paulo, SP, Brazil; 4) Unidade de Hepatologia Peditrica, Instituto da Crianca do Hospital das Clinicas da FMUSP, So Paulo, SP, Brazil.

INTRODUCTION The authors are members of the Sao Paulo State Expert Committee on Gaucher Disease (GD) [Sao Paulo, SP, Brazil] and now report demographic data and the evolution of patients treated in Sao Paulo State in accordance with the Clinical Protocol and Therapeutic Guidelines for GD Management created by the Brazilian Ministry of Health, www.saude.gov.br, Internal Regulation SAS/MS 449 of July 8, 2002. **METHODS** One hundred and thirty patients were evaluated in May 2004. One hundred and thirteen of them were on Enzyme Replacement Therapy (ERT) with imiglucerase, 75% of them (85/113) over 12 years of age. GD manifestations were investigated in patients over 12 years of age who were given doses of 15-30 U/kg every two weeks according to the Health Ministry (MS) Protocol from August 2002 to November 2003. **OBJECTIVES** To present the evolution of patients aged over 12 years who had their ERT doses reduced on account of being treated according the above-mentioned protocol. **RESULTS** Of the 85 patients aged over 12 years, the ERT average dose was of 37.6 U/kg/2 weeks (dose range 13-62 U/kg/2 weeks). Of the 19 patients who received doses of 13-20 U/kg/2 weeks it was noticed that 4 cases progressed with no pain and were maintained on low doses, 14 cases reported bone pain again, one of them with bone infarct, and one case reported painless lytic injury. Impaired patients were given higher doses up to 60 U/kg/2 weeks. Of the patients who received doses of 20-30 U/kg/2 weeks, 1 case progressed with no pain and was maintained on the same doses, 6 cases reported bone pain and 1 case reported a Parkinsonism condition. For the latter, the dose had to be increased up to 60 U/kg/2 weeks. **CONCLUSION** The authors reached the conclusion that ERT doses must be customized and that low doses (15 U/kg/2 weeks) are often insufficient to prevent GD complications.

Comparison of intravenous versus subcutaneous enzyme replacement therapy in the acid sphingomyelinase deficient mouse. *E. Schuchman*¹, *H. Jin*^{1,2}, *X. He*¹, *C. Simonaro*¹. 1) Dept Human Genetics, Mount Sinai Sch Medicine, New York, NY; 2) College of Veterinary Medicine, Kyungpook National Univ, Korea.

An inherited deficiency of acid sphingomyelinase (ASM) activity leads to neuronopathic and non-neuronopathic forms of Niemann-Pick disease (NPD). In all ASM deficient NPD patients, the spleen, liver and lungs represent major sites of pathology that must be targeted for therapy. Preclinical studies in the mouse model of ASM deficiency (ASMKO mice) have revealed that intravenous, bolus injection of recombinant ASM could prevent or reverse most of the disease manifestations in the spleen and liver, and could also prevent the infiltration of inflammatory cells into the lungs (Miranda *et al.*, FASEB J., 2000). However, despite these positive results, intravenous enzyme replacement therapy (ERT) in human patients is limited by the requirement for life-long enzyme infusions and high cost. We have therefore used the ASMKO mouse model to compare the efficacy of intravenous to subcutaneous ERT. For subcutaneous administration, a gas propelled Bioinjector apparatus was used. Age-matched ASMKO animals were given equivalent doses (1 mg/kg, once per week) of recombinant ASM intravenously or subcutaneously, and the efficacy of the therapy and pharmacodynamics were compared. Following enzyme injection, the peak amount of circulating ASM activity achieved by either delivery method was equivalent, although the release of this activity into the circulation was significantly slower when administered subcutaneously. The tissue distribution and efficacy also were similar, although slightly more activity was delivered to non-hepatic tissues by subcutaneous injection. Thus, subcutaneous ERT is a viable alternative to intravenous ERT, and may be considered for some lysosomal storage disorders where the safety profile is compatible with home administration.

Functional complementation of mammalian gulonolactone oxidase (GULO) deficiency using a recombinant adenovirus. *D. Sieron*¹, *M. Ha*¹, *F. Graham*^{1,2}, *J. Rosenfeld*², *S. Igdoura*^{1,2}, *H. Schellhorn*^{1,2}. 1) Department of Biology; 2) Department of Pathology and Molecular Medicine McMaster University. Hamilton, Ontario, L8S 4K1 CANADA.

Though most animals produce ascorbic acid, humans and guinea pigs are unable to produce this vitamin due to an inactivating mutation of the L-gulonolactone oxidase (GULO) gene, encoding the terminal enzyme in the biosynthetic pathway of vitamin C. A regular vitamin C supply from various foods is therefore essential to the animals bearing the genetic defect to maintain life. Prolonged vitamin C deficiency can result in scurvy, an acute condition resulting from inadequate collagen production. As an antioxidant, ascorbic acid may potentially reduce free radical accumulation and therefore protect against cancer and other oxidant-related diseases. The current reference dietary intake (RDI, formerly RDA) of ~90mg per day is likely adequate for protection against scurvy, however is probably far below ascorbic acid levels required for efficient antioxidant function. To correct the metabolic defect in mammalian cells by genetic complementation, we tested the efficacy of a recombinant adenovirus which transiently expresses the GULO gene (AdGULO1), in vitro in human carcinoma cell line (A549) and in vivo using guinea pigs. To ensure that deficient cells can be efficiently rescued by expressing the recombinant enzyme, a human lung carcinoma cell line (A549), that is non-permissive for replication, was infected with the GULO-encoding adenovirus and a mock virus control. The AdGULO1-infected, but not mock-infected, cells were proficient in synthesizing ascorbic acid indicating efficient expression from the viral construct. Animals maintained on vitamin C deficient diets that were infected with AdGULO1 exhibited reduced weight loss (a symptom of scurvy) than animals injected with a knock out version of the virus. We are currently investigating if high intracellular levels ascorbic acid can provide effective protection during oxidative stress.

Therapeutic Goals in the Treatment of Type 1 Gaucher Disease. *N.J. Weinreb¹, G.M. Pastores², H. Aerts³, G. Andria⁴, T.M. Cox⁵, M. Giral⁶, G.A. Grabowski⁷, P.K. Mistry⁸, A. Tylki-Szymanska⁹.* 1) University Research Foundation for Lysosomal Storage Diseases, Coral Springs, FL; 2) New York University School of Medicine, New York, NY; 3) Academic Medical Centre, Amsterdam, the Netherlands; 4) Federico II University, Naples, Italy; 5) Addenbrookes Hospital, Cambridge, UK; 6) Hospital Miguel Servet, Zaragoza, Spain; 7) Childrens Hospital Research Foundation, Cincinnati, OH; 8) Yale University School of Medicine, New Haven, CT; 9) The Childrens Memorial Health Institute, Warsaw, Poland.

Many type 1 Gaucher disease (GD) manifestations are reversed or relieved with regular infusions of enzyme replacement therapy (ERT), the internationally accepted standard of care. Treatment initiation and assessment must be tailored to the individual patients needs, as presentation, severity, and progression vary greatly. Because evidence-based, organ-system-specific, individualized therapeutic goals are needed, an international panel of physicians with extensive clinical expertise in type 1 GD reviewed ICGG Gaucher Registry findings and relevant published literature and created a comprehensive set of organ-system-based therapeutic goals to guide individualized therapy. These goals assume that optimal treatment addresses the entire disease burden, the baseline severity of disease in each organ system, and the potential for irreversible pathology. A comprehensive initial evaluation of all organ systems and manifestations is essential for defining goals. Guidelines for each goal include the extent of treatment response and a timeframe for achievement. Achievement and continuity of the therapeutic goals are determined from a regular monitoring schedule for each disease compartment. Treatment success requires attainment and maintenance of goals for all disease compartments within the predicted timeframes. Provision of therapeutic goals to patients may promote compliance and prevent potentially harmful treatment interruptions. As used by experienced physicians and allied health professionals, this individualized, goal-directed approach is proposed to be the most effective way to improve and preserve health and function in patients with type 1 GD.

A Phase I/II Safety Study of DRX008A Glucocerebrosidase Replacement Therapy in patients with type I Gaucher disease. *A. Zimran¹, D. Elstein¹, K.S. Loveday², R.J. Fram².* 1) Gaucher Clinic, Shaare Zedek Medical Ctr, Jerusalem, Israel; 2) Transkaryotic Therapies, Inc. Cambridge, MA, USA.

Gaucher disease is an autosomal recessive lysosomal storage disease caused by deficiency of the lysosomal enzyme - glucocerebrosidase. DRX008A is a human -glucocerebrosidase, a monomeric glycoprotein, produced by gene activation in a human cell line. Glycosylation of DRX008A is altered during cell culture resulting in the secretion of a protein containing predominantly high mannose type glycans. This allows DRX008A to be effectively incorporated into the phagocytic cells via mannose receptors that target the protein to cells of the reticuloendothelial system (predominantly macrophages, the target cells in Gaucher disease), where it can function to degrade accumulated glucocerebroside. DRX008A has been well tolerated in acute and repeat dose toxicity studies in rats and monkeys. The data from the non-clinical program indicated that DRX008A was safe to administer to patients with Gaucher disease as enzyme replacement therapy. The current trial is an open-label, Phase I/II safety study. The objectives of the trial are to evaluate the safety of purified DRX008A when administered at a dose of 60 U/kg IV every two weeks for a total of 40 weeks (20 IV infusions) to patients with type 1 Gaucher disease. The secondary objectives are: first, to evaluate the clinical activity of DRX008A in these patients, as measured by improvement in hematological parameters and reductions in liver and spleen volume; and second, to assess pharmacokinetics. In order to enhance patient safety, a staggered dose escalation of DRX008A was performed in a cohort of 3 patients. Patients received 15 U/kg, 30U/kg, and then 60 U/kg using an intra-patient dose escalation. Patients were infused every two weeks. After dose escalation was completed, patients in this cohort as well as an additional 9 patients are treated at 60 U/kg IV every two weeks for a total of 20 IV infusions. Preliminary safety data and pharmacokinetics will be presented from the patients enrolled in this trial. Results from this study will inform the design of future clinical studies of DRX008A in patients with Gaucher disease.

Therapeutic antisense-induced exon skipping for Duchenne muscular dystrophy. A. Aartsma-Rus¹, A. Janson¹, G. Platenburg², M. Bremmer-Bout¹, J.T. Den Dunnen¹, J.C.T. Van Deutekom¹, G-J.B. Van Ommen¹. 1) LUMC, Leiden, the Netherlands; 2) Prosensa, Leiden, the Netherlands.

The severe Duchenne muscular dystrophy (DMD) is mostly caused by frame disrupting mutations in the dystrophin gene, which result in non-functional proteins. Mutations that keep the reading frame intact give rise to internally deleted, semi-functional dystrophins and result in the milder Becker muscular dystrophy (BMD). Antisense oligonucleotides (AONs) have the potential to modulate the pre-mRNA splicing such that a specific exon is skipped. As a result, the reading frame can be restored, which allows the synthesis of a BMD-like dystrophin.

We have recently demonstrated the skipping of 20 different human DMD exons using 2'-O-methyl RNA AONs with a full-length phosphorothioate backbone (2OMePS), and confirmed the therapeutic applicability of this strategy in cultures from 10 different DMD patients. We now compare the efficacy and efficiency of 2OMePS AONs to morpholino, locked nucleic acid (LNA) and peptide nucleic acid (PNA) AONs. While LNA was most efficient in inducing exon skipping, we find that it acts in a less sequence-specific manner, probably due to its extremely high RNA affinity. Awaiting further improvements in oligochemistry, we therefore consider 2OMePS AONs currently the most favourable compounds.

To facilitate clinical application, multiple *in vivo* studies in animal models are ongoing to develop safe and efficient AON-delivery methods. However, as exon skipping is a sequence-specific therapy, it is desirable to directly target the human DMD gene. We have therefore set up sequence-specific human exon skipping *in vivo* in transgenic mice carrying the an intact copy of the full-length 2.5 Mb human gene (*hDMD*). We injected 2OMePS AONs targeting human exons 44, 46 and 49 into the *m. gastrocnemicus* of *hDMD* mice, and showed skipping of the human (but not the murine) exons, which persisted for at least 28 days. Based on pre-clinical data obtained by our group and others, we are currently setting up a clinical trial aiming at local dystrophin restoration following intramuscular injections of exon 46 and 51 specific AONs.

Prolonged Phenotypic Correction of Canine Hemophilia B Following Systemic Administration of a Helper-Dependent Ad Vector Encoding Coagulation Factor IX. *N. Brunetti-Pierri¹, D. Palmer¹, T.C. Nichols², A.L. Beaudet¹, P. Ng¹.* 1) Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC.

Helper-dependent adenoviral vectors (HDAd) offer tremendous potential for liver-directed gene therapy because they can transduce hepatocytes following systemic delivery to direct high-level, long-term transgene expression with negligible chronic toxicity. In this study we have evaluated the potential of liver-directed, HDAd-mediated hemophilia B gene therapy in a large animal model. Two hemophilia B dogs were injected intravenously with 3×10^{12} vp/kg of a HDAd encoding canine coagulation factor IX (FIX) under liver-restricted expression. Following vector injection, the whole blood clotting time (WBCT) for both dogs declined from >60 minutes to 11-19 minutes (normal range: 8-12 min) for a period of up to one year. aPTT decreased from >150 seconds to 48.5-51 seconds (normal range: 28-35 seconds) by 24 days post-injection and peak levels of FIX activity of 34.1% and 129% were detected at 12-14 days post-injection and declined by about 4 months to 2% and 5% but then remained stable at these levels for more than 7 months. FIX antigen levels stabilized at ~200 ng/ml for 12 months. Importantly, whereas untreated hemophilia B dogs suffer five to six spontaneous bleeds per year, the two treated dogs have suffered no such bleeds after vector injection. Systemic vector administration was accompanied by only mild and transient elevation of serum transaminases and thrombocytopenia with no evidence of chronic toxicity. These results support the potential for HDAd-mediated, liver-directed gene therapy for hemophilia B.

Gene transfer in the Sandhoff mouse model using AAV and lentiviral vectors. *C. Caillaud*¹, *A. Arfi*¹, *C. Bourgoin*¹, *C. Emiliani*², *L. Basso*³, *B. Tancini*², *S. Sonnino*³, *L. Poenaru*¹. 1) Dept Genetique, Institut Cochin, Paris, France; 2) Dept of Cellular and Molecular Biology, Perugia, Italy; 3) Dept of Medical Chemistry, Biochemistry and Biotechnology, Milan, Italy.

Sandhoff disease is an autosomal recessive neurodegenerative disorder characterized by the intralysosomal accumulation of GM2 ganglioside. It is due to mutations in the HEXB gene encoding the hexosaminidases -chain and results in a hexosaminidase A () and B () deficiency. This neurolipidosis has a dramatic evolution leading to early death.

In order to test the feasibility of gene transfer methods in this model, AAV vectors containing the human HEXA and HEXB cDNAs under the control of the CAG promoter were constructed. AAV-HEXB was injected alone into the brain of *hexb*^{-/-} neonates. Histological staining using an artificial substrate coupled with a naphthol residue showed a high enzymatic activity in widely diffuse areas. Hexosaminidases activity was restored to the normal level in the whole brain, but Hex A level remained low. The coadministration of both HEXA and HEXB vectors in the CNS allowed to overexpress both subunits, leading to a much higher Hex A activity. More importantly, a strong decrease in the GM2 storage was observed in the injected hemisphere, demonstrating that therapeutic levels of Hex A can be reached by supplying both subunits.

Mono and bicistronic lentiviral vectors (SIV) containing the HEXA and HEXB cDNAs under the control of the CMV promoter were also constructed. Functional tests of these vectors were performed *ex vivo* on Sandhoff fibroblasts. The bicistronic SIV.HEXA-IRES-HEXB was able to generate a massive restoration of the hexosaminidases activity, reaching one to threefold the normal level. The GM2 metabolism was restored not only in transduced cells, but also in cross-corrected cells, demonstrating a correct targeting of recombinant enzymes to the lysosome. This lentiviral vector will now be tested *in vivo* in *hexb*^{-/-} mice, by direct injection in the central nervous system using stereotactic methods.

Gene therapy for MPS II: strengthening Iduronate sulfatase enzymatic activity through the action of the Sulfatase Modifying Factor 1. *M. Cosma¹, S. Pepe¹, A. Auricchio¹, A. D'Azzo², A. Ballabio¹.* 1) TIGEM, via P. Castellino, 111, 80131 Naples, Italy; 2) St. Jude Children's Research Hospital, 332 North Lauderdale Street, Memphis, TN 38105, USA.

Sulfatases are a highly conserved family of proteins that cleave sulfate esters from a wide range of substrates including glycosaminoglycans, sulfolipids, and steroid sulfates. The importance of sulfatases in human metabolism is underscored by the presence of at least eight human monogenic diseases including five mucopolysaccharidoses (MPS) caused by the deficiency of individual sulfatase activities. MPS II (Hunter syndrome) is a lysosomal storage disorder due to the deficit of the Iduronate sulfatase (IDS). The Hunter mouse model exhibits many of the characteristics of MPS II, including skeletal abnormalities, elevated accumulation of GAGs in the urine and many organs. The enzymatic activity of sulfatases, including the Iduronate sulfatase, requires a post-translational modification at the active site that transforms a cysteine into formylglycine. We have recently identified the gene SUMF1 (Sulfatase modifying factor 1), responsible for this post-translational modification, which is inactive in the Multiple Sulfatase Deficiency (MSD). SUMF1 strikingly activates all human sulfatases. In order to develop an effective gene therapy to rescue the phenotype of MPSII mice we are injecting group of KO mice either with IDS alone or simultaneously with SUMF1 using adeno-associated vectors. This protocol will test whether the delivery of the two genes results in an effective treatment of this deficit by increasing IDS activity *in vivo*. Our final goal is to engineer one tissue (muscle or liver) to secrete active IDS into the bloodstream to systematically cross-correct the enzymatic deficiencies in various tissues. Preliminary results already show clearance of GAGs in the urine and in the transduced and not transduced tissues of treated mice. We are also characterizing the CNS defects of the MPSII mice and we plan to rescue the central nervous system phenotype of MPS II mouse by delivering IDS with/without SUMF1 in specific area of the brain.

Multimeric hammerhead ribozymes show specific and efficient cleavage of glycine substitutions within the triple helical domain of Col1A1. Z. Lada¹, D. Basel¹, M.W. Kilpatrick¹, R.J. Wenstrup², P. Tsipouras¹. 1) Genetics & Dev Biol, UConn Health Ctr, Farmington, CT; 2) Cincinnati Childrens Hospital Research Foundation, Cincinnati, OH.

Hammerhead ribozymes are small catalytic RNA molecules that can be engineered to cleave a target RNA in a sequence-dependent manner. The dominant-negative effect exerted by the mutant collagen in Osteogenesis Imperfecta (OI) has been the focus of our previous studies on ribozyme targeting of the abnormal protein to restore the normal phenotype. Herein, the pMG155 Col1A1 minigene, which causes the OI phenotype in both cell lines and mouse models was further engineered to incorporate a murine triple helical domain derived from the Col1A2 gene. This construct, pMG155A2, was then mutated to introduce 3 distinct, ribozyme targetable, glycine substitutions. Gly(A)Rz targets a G to C mutation that produces a cuc ribozyme cleavage site, Gly(B)Rz a G to C mutation that produces an auc cleavage site and Gly(C)Rz a G to T mutation that produces a guc cleavage site. In vitro studies showed that ribozymes directed at these single base changes are specific in that they cleave the substitutions but not the wild-type sequence. To ensure sufficiently high levels of ribozyme expression within a cellular system, multimers comprising tandem repeats of the ribozyme subunit were constructed and shown to cis-cleave to the active monomeric subunits. We then constructed heteromultimers of the ribozyme subunits to target the triple helical domain of Col1A2 containing all 3 of the glycine mutations. Initial results suggest that the multimers cleave the target more efficiently than the monomers and that the heteromultimers cleave with even greater efficiency. Although multiple targets would not exist in a single patient with OI, several glycine substitutions appear with higher frequency. This approach allows for the design of a heteromultimeric ribozyme targeting several of the most commonly occurring collagen mutations as a potential therapeutic agent. It also has potential use in a system where it is beneficial to target multiple sites e.g. HIV or other viruses where specificity and efficiency are both important factors.

Correction of Maternal PKU Syndrome in the *Pah*^{enu2} Missense Mutant Mouse by r-AAV Mediated Gene Therapy. *P.J. Laipis*¹, *C.E. Charron*¹, *J.E. Embury*¹, *O.P. Perera*¹, *S.L. Porvasnik*², *C.R. Fields*¹, *R.T. Zori*². 1) Dept Biochem & Molecular Biol, Univ Florida, Gainesville, FL; 2) Dept Pediatrics, Univ Florida, Gainesville, FL.

Current treatment for PKU is strict lifelong restriction of phenylalanine (Phe) in the diet. A complication of poor adult compliance is a high risk of Maternal PKU Syndrome in offspring of PKU women who are unable to control Phe levels during pregnancy. We had achieved therapeutic reduction of serum Phe levels in male *Pah*^{enu2} mice using Type 2 AAV vectors carrying the mouse phenylalanine hydroxylase gene (rAAV-mPAH). Portal vein injection of a new rAAV-mPAH vector with a Woodchuck Hepatitis Virus post-transcriptional response element (WPRE) into male *Pah*^{enu2} mice reduced serum Phe to therapeutic levels (<0.5 mM) at 10-fold lower vector doses ($\sim 5 \times 10^9$ IU) and to physiological levels (0.05-0.1 mM) at $\sim 2.5 \times 10^{10}$ IU. While female *Pah*^{enu2} mice did not respond to doses effective in males, therapeutic reductions were seen at doses of $\sim 1.5 \times 10^{11}$ IU.

Vectors were also packaged in AAV Type 5 capsids. Type 5 mPAH-WPRE vector ($\sim 7 \times 10^{13}$ particles) resulted in physiological Phe levels in female *Pah*^{enu2} mice. Treated females delivered viable pups when mated with male *Pah*^{enu2} mice. The obligate PKU pups developed normally. Females given lower vector amounts, with therapeutic serum Phe levels (0.15 to 0.5 mM), also became pregnant. Pups died neonatally or failed to thrive post birth. Interestingly, serum Phe levels in mothers rose dramatically during lactation but fell to pre-birth levels at weaning. Finally, while the WPRE sequence increased vector efficiency, we observed liver abnormalities and increases in serum Phe levels starting about 20 weeks post vector delivery. The relative inefficiency of gene therapy in the *Pah*^{enu2} PKU mouse model suggests that dominant-negative interactions between vector-derived normal subunits and endogenous PAH missense subunits are occurring in the PAH tetramer (see Charron et.al. abstract). Our results emphasize the importance of examining both missense and null mutations when evaluating the effectiveness of gene therapy for human genetic disease.

Long-term clinical correction and vector genome persistence following gene therapy of hemophilia A dogs with a helper-dependent adenoviral vector. *W. McCormack Jr¹, T.K. Bertin¹, K. Ubhayakar¹, M. Guenther¹, V.P. Mane¹, P. Ng¹, D. Palmer¹, T.C. Nichols², M. Finegold³, A. Beaudet¹, B. Lee^{1,4}.* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, NC; 3) Department of Pathology, Texas Children's Hospital, Houston, TX; 4) Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX.

Helper-dependent adenoviral vectors (HDV) offer the advantage of a large cloning capacity, reduced toxicity and extremely efficient transduction of target tissues. These vectors remain episomal in host target cells limiting the possibility of insertional mutagenesis but potentially reducing the duration of transgene expression due to vector genome loss. In this experiment, we treated two hemophilia A dogs from the colony at the University of North Carolina with a HDV encoding canine blood coagulation factor eight (cFVIII). One animal received 1×10^{12} viral particles per kilogram (vp/kg) and a second received 3×10^{12} vp/kg. The whole blood clotting time (WBCT) in both of the animals was significantly improved. In the higher dose animal, this improvement has lasted for over a year. However, the plasma level of cFVIII declined from peaks at two weeks post-treatment of 1.3% normal in the low dose animal and 70% normal in the high dose animal to less than 1% at 2 months in both. To determine the reason for this, a third animal was injected with 3×10^{12} vp/kg and underwent survival liver biopsy at days 18 and 79 after vector administration correlating with the high (35% normal) and low (<1% normal) levels of cFVIII in the plasma. Genomic DNA was isolated from these biopsy samples. Southern analysis, Real-time PCR and semi-quantitative PCR indicate that there was at most a minimal decline in vector genome from the early to the late time point. These data suggest that vector genome loss alone is not sufficient to account for the decline of plasma cFVIII levels.

High efficiency transduction and long-term transgene expression by delivering helper-dependent adenoviral vectors into the surgically isolated liver of nonhuman primates. *P. Ng¹, T. Ng², D. Iannitti², W. Cioffi², D. Palmer¹, A.L. Beaudet¹, M. Finegold³, K. Rice⁴, D. Carey⁴, N. Brunetti-Pierri¹.* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Surgery, Brown Medical School, Providence, RI; 3) Department of Pathology, Baylor College of Medicine, Houston, TX; 4) Southwest Foundation for Biomedical Research, San Antonio, TX.

Helper-dependent adenoviral vectors (HDAd) are attractive vectors for liver-directed gene therapy because they can mediate sustained, high level transgene expression with negligible long-term toxicity. However, high vector doses are required to achieve efficient hepatic transduction by peripheral intravenous injection due to a nonlinear dose response. Unfortunately, such high systemic doses result in vector dissemination and dose-dependent acute toxicity. We hypothesize that this obstacle can be surmounted by delivering the vector exclusively to the liver. To test this hypothesis, we injected HDAd directly into the surgically isolated liver via the portal vein in nonhuman primates. Total hepatic isolation was achieved by occluding hepatic inflow from the portal vein and hepatic artery and by occluding outflow at the vena cava. Prior to total hepatic isolation, saline was infused into the portal vein to flush blood out of the liver. The vector was then injected via the portal vein and allowed to dwell for 30 min. Unabsorbed vector was then removed from the liver to minimize systemic dissemination by infusing saline into the portal vein and collecting the wash-out from a vena cava catheter. Our results revealed that the surgical procedure was well tolerated in nonhuman primates and that significantly higher hepatic transduction efficiencies can be achieved with relatively low vector doses compared to peripheral intravenous injection. Long-term, high level transgene expression was also observed with only minimal and transient toxicities. This approach may increase the safety and efficacy of HDAd-mediated, liver-directed gene therapy by minimizing the dose required to achieve efficient hepatic transduction and by minimizing systemic vector dissemination.

RNAi induction in mammalian neurons and muscle cells. *K. Omi^{1,2}, N. Sago^{1,2}, K. Tokunaga¹, H. Hohjoh²*. 1) Dept Human Genetics, Univ Tokyo, Tokyo, Japan; 2) National Institute of Neuroscience, NCNP, Tokyo, Japan.

RNA interference (RNAi) is the process of sequence-specific posttranscriptional gene silencing mediated by 21-25 nucleotide (nt) small interfering RNA (siRNA) duplexes homologous to the silenced genes. The application of RNAi appears to be expanding to various fields including medical science and pharmacology. An important point in the application of RNAi seems to be the persistence of RNAi activity in cells, particularly in mammalian cells. The effect of RNAi induced by synthetic siRNA duplexes on proliferating mammalian cells appears to be temporary, i.e., it appears to persist for approximately 3-4 days after its induction. Here we show that the RNAi activity induced by synthetic 21-nt siRNA duplex in post mitotic neurons, mouse primary hippocampal neurons and neurons that differentiated from mouse embryonal carcinoma P19 cells, lasts for at least three weeks. We also examined the properties of RNAi during myogenic differentiation of C2C12 cell, a mouse myoblast cell line. The result indicate that a strong RNAi activity was detectable by day 7 after RNAi induction, when morphological changes of C2C12 cells into myotubes appeared to be completed; thereafter, the cells gradually lost the RNAi activity and lost most of the activity by day 21 after the induction. Therefore, our present observations suggest that there is a significant difference in the duration of RNAi activity between neuron and muscle, both of which are terminally differentiated and cell cycle-arrested cells. Since neither muscle nor neuron probably undergoes a decrease in the number of functional RISCs by cell division, it is possible that the stability of functional RISCs could differ between neuron and muscle.

Recombinant Adeno-associated Virus-Mediated Gene Transfer in Homocystinuria Mice. *E.S. Park¹, H.J. Oh², S.C. Jung², J.S. Lee^{1,3}*. 1) Brain Korea 21 Project for Medical Science, Yonsei Univ, Seoul, Korea; 2) Division of Genetic disease, Department of Biomedical Sciences, National Institute of Health, Seoul 122-701, Korea; 3) Department of Clinical Genetics, Yonsei University College of Medicine. Seoul 120-752, Korea.

Homocystinuria is a metabolic disorder caused by a deficiency of cystathionine -synthase (CBS). Patients with homocystinuria show mental retardation, lens dislocation, vascular disease with life-threatening thromboembolisms, skeletal deformities. Generally, the major treatments for CBS deficiency include pharmacologic doses of pyridoxine or dietary restriction of methionine. However, there is no effective treatment for this disease up to now. Gene therapy is an attractive novel approach to treatment of homocystinuria. We constructed recombinant adeno-associated virus vector carrying the human CBS cDNA (rAAV-hCBS) and administered to CBS^{-/-} mice by the intramuscular (IM) and intraperitoneal (IP) injection. IP injection was more efficient than IM injection for prolongation of lives and reduction of plasma homocysteine levels. After 2 weeks of gene transfer by IP injection, serum homocysteine level was significantly decreased in treated mice compared with the age-matched controls. Also, the life span was extended about 1.5 times and increased expression of CBS gene was observed by immunohistochemical staining in liver of treated CBS^{-/-} mice. Here, we show that a CBS gene transfer by intra peritoneal injection brought about elongation of life span and reduction of plasma homocysteine level. These results demonstrate possibility and the efficacy of gene therapy by AAV gene transfer in homocystinuria mice.

Significant increase in life span of mouse models of globoid cell leukodystrophy after AAV-mediated transfer of galactocerebrosidase cDNA into brain. *M.A. Rafi¹, H.Z. Rao¹, M. Curtis², M.A. Passini³, J.H. Wolfe⁴, D.A. Wenger¹.*
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Globoid cell leukodystrophy (GLD) or Krabbe disease is a neurodegenerative disorder caused by the deficiency of the lysosomal enzyme galactocerebrosidase (GALC). This deficiency results in the accumulation of certain galactolipids including psychosine which is cytotoxic for myelin-producing cells. Twitcher, the naturally occurring mouse model of GLD with the W339X mutation in the C57BL background and mice with the same mutation in a hybrid background of 129SVJ and FVB/N were used in this study. After cloning the mouse GALC cDNA into a type-2 adeno-associated viral genome, and packaging into the AAV1 serotype (AAV2/1-mGALC), viral particles were injected intra-ventricularly or intra-cranially into the brains of the neonatal mice (3×10^{10} viral particles/injection). The treated mice had milder symptoms than untreated mice and remained active most of their lives. Treated mice had a 70% increase in their life span when virus was delivered bilaterally. Analysis of their brains demonstrated a widespread distribution of GALC activity with highest levels near the sites of intra-cranial injection. Frozen sections were stained with anti-GALC antibody, and cells expressing high levels of GALC antigen had bright perinuclear staining. These cells were located mostly in hippocampal, septal, and cortical regions. GALC antigen and activity were still detectable 80 days after viral injection but the strongest signal was detected 2 weeks after injection. Light microscopy of the treated animals 55 days after injection showed near normal myelination in corpus callosum. However, there was more demyelination still present in the cerebellum. These studies demonstrate the ability of AAV2/1-mGALC to deliver GALC activity throughout the brain. While this results in a significant increase in the life span of these mice, there may be other factors needed to completely correct this disorder in animal models and human patients.

Long-term expression of human -galactosidase A in Fabry mice by neonatal lentiviral gene transfer. M.

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Fabry disease is a lysosomal storage disorders (LSD) caused by a deficiency of -galactosidase A (-gal A). Fabry patients respond to enzyme replacement therapy, however frequent infusions are required, strong antibody responses occur in some patients, and the long-term benefits in key organs have not been established. Stable gene augmentation by direct *in vivo* administration of vectors offers considerable potential as an effective, long-term, and relatively non-invasive therapeutic approach. However, *de novo* expression of a corrective, although foreign, protein may result in immune responses that eliminate the transduced cells. Neonates have immature immune systems and treatment at this early stage may also prevent irreversible organ damage. One strategy toward this aim is neonatal gene transfer. Here we explore the use of a single intravenous injection of a VSV-g pseudotyped lentiviral vector (LV) that encodes the -gal A cDNA. Neonatal Fabry mice were injected through the temporal vein with 100 μ l of concentrated LV (1×10^6 infectious particles) at 2 to 3 days after birth. In plasma from injected Fabry mice, we observed sustained and stable -gal A activity (average 39.226.5 of wild type enzyme levels, n=12) 24 weeks post-injection. To determine the bio-distribution of vector expression in live animals, we also used a LV that encodes the luciferase cDNA. We observed strong bio-luminescence systemically 11 weeks after injection. Relevant organs will be assayed from all injected mice for -gal A activity, luciferase expression and substrate Gb3 levels. These results suggest that this LV delivery system may offer significant long-term therapeutic advantages for LSDs and other gene therapy applications.

Molecular analysis of CYP21 gene in 30 patients with 21-hydroxylase deficiency and its prenatal diagnosis in Northeastern Iran. *M.R. Abbaszadegan¹, R. Vakili², A.R. Baradaran-Heravi^{1,2}, B. Barid-Fatehi^{1,2}, Z. Vahedian¹, N. Ghaemi².* 1) Division of Human Genetics, Immunology Research Center, Bu-Ali research institute, Mashhad University of Medical Sciences (MUMS), Mashhad, Iran; 2) Division of Pediatric Endocrinology, MUMS, Mashhad, Iran.

OBJECTIVES: A rapid and convenient approach for the most common CYP21 mutations in patients with congenital adrenal hyperplasia (CAH) with classical forms of 21-hydroxylase (21-OH) deficiency was used. A new semi-quantitative strategy for detection of del 8-bp was designed. These procedures were used in prenatal diagnosis and the genotype-phenotype correlation in province of Khorasan, northeastern Iran. **DESIGN:** Molecular analysis of the CYP21 gene for 9 most common mutations (del-CYP21, del-8bp, i2g, R356W, I172N, P30L, E6 cluster, Q318, V281L) were performed in 30 CAH patients and two fetuses. **METHODS:** Amplification-created restriction sites (ACRS), allele-specific PCR and semi quantitative PCR. **RESULTS:** We characterized 90% of the CAH chromosomes. The most frequent mutation in CYP21 gene was del-CYP21 (25%). The most frequent point mutations were I172N (22%) and intron 2 splice mutation (15%). There were no R356W and P30L mutations in our patients. We detected more than 2 mutations in 6 patients, for instance one patient was homozygous for both V281L and I172N. We also successfully applied these methods for prenatal diagnosis in two families. A correlation between the genotype and the clinical phenotype was observed similar to previous reports. The rate of homozygosity (50%) was greater than what was predicted due to the inheritance pattern (autosomal recessive) of the disease and it was due to the higher rate of parental consanguinity in our population. **CONCLUSIONS:** These procedures proved to be sensitive and rapid for the detection of the most common known mutations in the CYP21 gene and useful for prenatal diagnosis. Increased 17-hydroxyprogesterone found in neonatal CAH screening can be confirmed by these mutational analysis.

Prenatal diagnosis of α -thalassemia, a small gene, a complex problem. *S. Zeinali*^{1,2}, *B. Azimifar*², *V. Lotfi*², *M. Masrou*², *P. Fouladi*², *M. Eram*², *Hosaini*². 1) Biotechnolog, Pasteur Institute of Iran, Pasteur St., Tehran, Iran; 2) Medical Genetics Laboratory of Dr. Zeinali, No 21, Leon Building, Bisotoon St., Fatemi Sq., Tehran, Iran.

α -thalassemia is the most prevalent genetic disorder in the world. In Iran there exist more than 15000 affected individuals. We are using different approaches to curve the birth of new cases. Premarital screening is compulsory. Those detected as carrier couple are counselled and then referred to one of the prenatal diagnosis centers (PND) in the network. By doing PND we have faced the reality and complexity of PND for α -thalassemia. Problems like simultaneous presence of α -thalassemia which complicates the blood indices parameters, inaccurate A2 results, Iron deficiency, deletion of the α -globin cluster, non-paternity, etc. have made PND a bit complicated. We are using direct mutation detection in conjunction with RFLP to minimize the risk of misdiagnosis. Direct DNA sequencing has made the diagnosis more reliable. Sometimes sequencing does not show any mutation. Then we may be puzzled in accepting that either there is no mutation or the sequencing may not be accurate. In other cases not all of the family members may be present for RFLP analysis. So far we have made more than 800 PND in our private lab for the past 4 years with only one mistake, which is below to the standard level but still too much for us and the family. We have advanced our facilities and techniques to minimize misdiagnosis by using techniques like gap PCR for α -globin gene deletion, for α -globin gene deletion and mutation, direct DNA sequencing, QRT PCR, RFLP, etc. The Iranian Experience in curving α -thalassemia both at premarital level as well as post marital by offering PND can be used by other neighbouring countries.

PLA2G7 gene polymorphisms and multiple sclerosis. *L. Provezza¹, M. Gomez-Lira¹, C. Terranova¹, M.D. Benedetti², S. Ottaviani², A. Salviati², P.F. Pignatti¹.* 1) Department of Mother and Child, Biology and Genetics, University of Verona, Verona, Italy; 2) Department of Neurological Science, University of Verona, Verona, Italy.

A marked increased of platelet-activating factor (PAF), in both plasma and CSF, has been observed in the active phase of relapsing/remitting form in multiple sclerosis (MS) patients with clear evidence of blood-brain barrier damage. The bioactivity of PAF is attenuated via its hydrolysis by PAF acetylhydrolase (PAF-AH, or lipoprotein-associated phospholipid A2, Lp-PLA2), and lower levels of Lp PLA2 activity have been reported in MS patients when compared to control individuals. We have evaluated the association of Lp-PLA2 gene (PLA2G7) polymorphisms with susceptibility to MS in a small case-control study in the north Italian population, including 139 MS patients and 139 sex matched healthy controls. A total of eight polymorphisms throughout the gene were analyzed: three exonic polymorphisms (Arg92His, Iso198Thr, and Val379Ala), a deletion polymorphism (1190-20_23delGATT) in intron 11, two highly polymorphic dinucleotide repeats (1134+31TGmAGn) in the 3' flanking region, and two SNPs (-402T>C and 209C>G) in the promoter region of the gene. No statistically significant difference in the frequency of alleles and genotypes was observed between patients and controls suggesting that sequence variations of the PLA2G7 gene do not play an important role in susceptibility to MS in this group of patients.

Retroviral gene transfer into autologous peripheral CD34 hematopoietic cells in a 12 year old with X-linked severe combined immunodeficiency. *J. Puck¹, J. Chinen¹, J. Davis¹, G. Linton², N. Theobald², M. Garolfalo², P. Woltz², H. Malech².* 1) NHGRI, NIH; 2) NIAID, NIH, Bethesda, MD.

X-linked severe combined immunodeficiency (XSCID), due to defects in the common gamma chain (gc) of receptors for IL-2 and other cytokines, is fatal without immunoreconstitution. Patients with no matched sibling donor for bone marrow transplant (BMT) receive haploidentical T-depleted BMTs, but often have incomplete engraftment. Correction of autologous hematopoietic stem cells by gene therapy has succeeded because lymphoid cells expressing functional gc undergo selective expansion. In a French trial, 9 of 10 XSCID infants had immune reconstitution after ex vivo gene therapy with retrovirus-transduced autologous CD34 cells. However, the 2 youngest, treated at 1 and 3 mo, developed T cell leukemias in which the LMO2 transcription factor was activated by nearby provirus insertions. While risks of gc gene transfer as primary treatment for XSCID continue to be evaluated, older boys who have failed BMTs are candidates for gene transfer as salvage treatment. An 11 y/o with no engraftment from prior parental T-depleted BMTs had low T cells, growth failure, infections and chronic GI and skin infiltration by autologous, dysregulated lymphocytes. He underwent G-CSF mobilization and apheresis to harvest CD34 cells, which were purified, cultured in fibronectin-coated bags (with SCF, Flt3 ligand, IL-3, IL-6, and TPO) and transduced for 4 d with high-titer GALV-MFGS-gc retroviral supernatant. 80 million cells/kg (80% CD34+; 40% provirus positive) were reinfused. Polyclonal total leukocyte marking was 1.4% and 2.3% at 1 and 2 mo, but undetectable at 3 mo, consistent with limited lifespan of myeloid progenitors. However, at 5 mo thymus size rose from 1.80 to 3.76 cc, and marking reappeared in lineages dependent on gc expression: 0.5% in T, 0.1% in NK and 0.05% in B cells. Within 2 mo of gene transfer the boy experienced sustained improvement in well-being with resolution of lifelong diarrhea and rash. Further follow-up will monitor retrovirus insertion sites and look for progressive in vivo expansion of corrected lymphoid cells. Gene therapy may benefit older, post-BMT patients with XSCID.

Computerized cognitive testing in patients with type I Gaucher disease: effects of enzyme replacement and substrate reduction. *D. Elstein¹, G. Doniger³, J. Guedalia², E. Simon³, A. Zimran¹.* 1) Gaucher Clinic, Shaare Zedek Medical Ctr, Jerusalem, Israel; 2) Neuropsychological Unit, Shaare Zedek Medical Ctr, Jerusalem, Israel; 3) NeuroTrax Corp., New York, NY.

During clinical trials using substrate reduction therapy (N-butyldeoxynojirimycin; OGT 918; ZAVESCA) for adults with type I Gaucher disease, the question of decreased cognitive function was raised. For long-term surveillance, Mindstreams (NeuroTrax Corp., New York, NY), a computerized system incorporating subtests specific to executive and visual-spatial cognitive function was used. Patients with type I Gaucher disease (N=84; mean age: 46.113.5; mean years of education: 14.52.6) were compared with age-, education-, and gender-matched controls (N=140). Mean Severity Score Index (SSI) in patients was 9.75.5, and 43 patients were homozygous for the 1226G mutation. Data in patients was normalized according to controls and fit to an IQ-style scale (mean: 100, SD: 15). Performance in untreated patients (N=26) was compared to that of patients receiving enzyme replacement (N=22) or Zavesca (N=36). Adjustment for SSI and genotype was by analysis of covariance (ANCOVA). Visual-spatial performance in patients on Zavesca was better than for untreated patients after adjustment for SSI ($F[1,54]=5.01$, $p=0.029$) and genotype ($F[1,53]=5.46$, $p=0.023$); this improvement was significantly greater for patients not homozygous for 1226G as compared to homozygotes [$F[1,53]=10.96$, $p=0.002$]. Visual-spatial performance in patients on enzyme was poorer than in those on Zavesca after adjustment for SSI ($F[1,51]=7.65$, $p=0.008$) and genotype ($F[1,51]=8.02$, $p=0.007$). Executive function performance was poorer in those on enzyme versus those on Zavesca after adjustment for SSI ($F[1,55]=6.61$, $p=0.013$), but also poorer in patients on enzyme as compared to those who were untreated after adjustment for SSI ($F[1,59]=6.406$, $p=0.014$). Global Cognitive Scores showed no treatment-mediated performance differences. These findings may allay fears of cognitive dysfunction due to substrate reduction, and may have implications for indications for Zavesca in type III Gaucher disease and neuronopathic forms of other lysosomal storage disorders.

Pharmacokinetics of Agalsidase Alfa in Fabry Patients with End-Stage Renal Disease. *J. Flaherty*¹, *G. Pastores*², *R. Schiffmann*³, *E. Boyd*⁴, *A. Whelan*⁵, *K. Loveday*¹. 1) Clinical Research, Transkaryotic Therapies, Inc, Cambridge, MA; 2) New York University, NY, NY; 3) NIH, Bethesda, MD; 4) Advanced Home Care, Asheville, NC; 5) Children's Hospital, St. Louis, MO.

PURPOSE: Evaluate pharmacokinetics (PK) of agalsidase alfa in Fabry patients with end-stage renal disease (ESRD).

METHODS: Serum samples were collected from patients who had received a kidney transplant (12 males and 1 female) and from hemodialysis patients (7 males and 1 female) following their first infusion of agalsidase alfa (Replagal, TKT, Inc., Cambridge, MA). Agalsidase alfa was administered after hemodialysis. Serum analysis and PK analysis were performed at TKT. Four patients were excluded from the comparative PK analysis since blood samples were collected from the same arm used for infusion of agalsidase alfa.

RESULTS: Agalsidase alfa had a biphasic serum elimination profile in the 17 analyzed patients. Serum profiles and PK parameters were similar to results from male Fabry patients who had not progressed to ESRD (non-renal failure). Mean serum clearance values were 2.5 mL/min/kg (18 non-renal failure males), 3.2 mL/min/kg (11 transplant patients) and 3.4 mL/min/kg (6 dialysis patients).

CONCLUSIONS: Since agalsidase alfa is primarily removed from serum and transported to cellular lysosomes via mannose-6-phosphate receptors, the effect of kidney function on serum clearance should be minimal. This expectation was met in these Fabry patients with ESRD. Serum clearance profiles and calculated clearance (mL/min/kg) were indistinguishable among transplant, renal dialysis, and non-renal failure patients. Based on the similarity of PK properties, tissue distribution in major tissues and organs is also expected to be comparable among these three classes of Fabry patients. Thus, the prescribed biweekly dose of 0.2 mg/kg (administered IV over 40 minutes) can be maintained in Fabry patients with ESRD.

Glycosaminoglycan Reduction in Response to Iduronate 2-Sulfatase Treatment in a Mouse Model of MPS II. *A.R. Garcia, K. Loveday, J. Pan, J.C. Lamsa.* Research, TKT, Cambridge, MA.

The IdS-KO mouse, in which a portion of the gene for iduronate 2-sulfatase (IdS) is deleted, develops elevated glycosaminoglycan (GAG) levels in tissue and urine and exhibits gross and microscopic pathology consistent with Hunter disease (MPS II). Adult IdS-KO mice were administered 1 mg/kg IdS intravenously (IV) as a single injection or at weekly, bi-weekly, and monthly intervals for up to 6 months. Cohorts of mice were sacrificed after 2, 3 or 6 months of treatment and the liver, spleen, kidney and heart collected for GAG analysis and histopathology. Vehicle treated IdS-KO and untreated wild type (WT) mice served as controls. GAG levels were measured by dimethylmethylene blue assay. Urinary GAGs were markedly reduced after the first injection. For weekly and biweekly injections, urinary GAG levels reached WT levels at 1 to 4 weeks and remained low. After a single injection or monthly injection, urinary GAG declined then rose gradually to vehicle control levels by 4 weeks. Two months after a single dose of IdS, GAG levels in all tissues were reduced 15% to 25% compared to vehicle controls, but remained considerably higher than WT animals. Tissue GAG levels continued to decline with increasing dose frequency. Through 3 months of treatment, weekly dosing consistently reduced GAGs to a greater degree than bi-weekly dosing. After 6 months, GAG levels continued to be significantly reduced to nearly WT levels, but there were no significant differences between weekly and bi-weekly dosing in terms of GAG reduction in liver, kidney, heart and spleen. In addition, liver weights were significantly reduced by either bi-weekly or weekly dosing for 6 months compared to vehicle controls. Microscopically, there was a marked reduction in vacuolization in all of the organs examined (liver, spleen, kidney and heart). We conclude that in the IdS-KO mouse, monthly dosing allows a rebound of GAG accumulation. Weekly or bi-weekly dosing effectively reduces GAG in urine and in major organs in this animal model of Hunter disease, although bi-weekly dosing takes longer to achieve maximal GAG reduction.

Substrate reduction therapy with miglustat for symptomatic type 1 Gaucher disease. *E.H. Kolodny, N.A. Barnett, G.M. Pastores.* Dept Neurology, New York Univ Medical Ctr, New York, NY.

OBJECTIVE: Miglustat (Zavesca) has recently been approved for the treatment of type 1 Gaucher Disease (GD). In the pivotal clinical trials with extension up to 24 months, miglustat significantly improved organomegaly, hematological parameters and levels of chitotriosidase. The study was designed to analyze the extended efficacy and safety of miglustat 100 mg PO tid. **METHOD:** 7 patients received miglustat for a minimum of 24-months. Comprehensive assessments were conducted, based on liver and spleen volume measurements, radiographic bone studies, and chitotriosidase (chito) activity, nerve conduction velocities (NCV), neuropsychological status and tremor. In addition, patients tolerability of miglustat was monitored. **RESULTS:** In the initial phase of the study, 10 patients received active drug; 3 patients discontinued due to adverse events (1 paresthesia, 1 flatulence, 1 lost to follow-up). At 24 months, the remaining 7 patients showed a mean percentage change in liver (-6%) and spleen (-15%) volumes, platelets (+23%), and chito (-25%); consistent with results of prior studies. The most common complaints were flatulence (n=10) and diarrhea (n=9); which resolved in the majority of patients. There was a mean decrease of 4.0 kg (6%) from baseline body weight. Tremor (n=4) and paresthesia (n=3) were reported early on but subsequently decreased over time. Notably there were no clinically relevant abnormalities observed on serial neurological examinations and tremors (observed at baseline in 2 of the 4 patients) were below threshold of detection by accelerometry. Additionally, no clinically meaningful changes in NCV over 24 months were noted, even among patients reporting paresthesia and no changes in cognitive function. **CONCLUSION:** Our 2-year study revealed stabilization and/or improvement in key clinical features of the disease, supportive of clinical proof-of-concept for the use of substrate reduction therapy in GD patients. No patient had emergent GD-related complications and there were no clinically significant abnormalities in neurological, neurophysiological or neuropsychological assessments.

Pharmacokinetics of Agalsidase Alfa in Pediatric Fabry Patients. *K.S. Loveday¹, M. Beck², C. Whybra², R. Schiffmann³, J. Clarke⁴, J. Flaherty¹.* 1) Research, Transkaryotic Therapies, Inc., Cambridge, MA; 2) Children's Hospital, University of Mainz, Mainz, Germany; 3) NIH, Bethesda, MD; 4) Hospital for Sick Children, Toronto, Canada.

PURPOSE: Evaluate pharmacokinetics (PK) of agalsidase alfa in pediatric Fabry patients.

METHODS: Serum samples were collected from 18 boys and 5 girls (6 to 18 years old) following their first IV infusion of agalsidase alfa (Replagal, TKT Inc. Cambridge, MA). Serum samples were analyzed for alpha-galactosidase A activity at TKT, and PK analysis was performed at TKT.

RESULTS: Agalsidase alfa had a biphasic serum elimination profile in all patients, and only small amounts were detected 8 hours after dosing. The maximum concentration in serum coincided with the end of the 40-minute infusion. Serum clearance (Cl) ranged from 2.0 to 9.4 mL/min/kg while V_{ss} (apparent volume of distribution) ranged from 9% to 26% body weight (BW). PK parameters in the 23 pediatric patients were similar to results from adult Fabry patients with the exception of the younger patients (6 - 11 years). Mean Cl for the 13 younger patients (4.2 mL/min/kg) was statistically increased compared to the adult value (2.3 mL/min/kg). Mean Cl for the 10 older pediatric patients (12 - 18 years) was 3.1 mL/min/kg; this value was intermediate between the clearance values for younger and adult Fabry patients. Mean V_{ss} was similar among younger and older pediatric patients and adults (15% - 16% BW).

CONCLUSIONS: Increased serum clearance in the 13 younger patients may be due to increased organ/body weight ratios. This would increase the number of M6P receptors/body weight leading to more rapid uptake into liver, spleen and other tissues. However, it is unlikely that the increased rate of serum clearance in the 13 younger patients significantly altered their overall tissue biodistribution of administered agalsidase alfa. Therefore, the recommended adult dose, 0.2 mg/kg, should not be altered to compensate for the more rapid serum clearance in younger pediatric patients.

NAG-Thiazoline a specific, efficient and safe chemical chaperone for the treatment of adult Tay-Sachs and Sandhoff diseases. *D. Mahuran¹, B. Rigat¹, S. Reid², H. Yeager¹, J. Clarke¹, S. Withers²*. 1) The Hospital for Sick Children, Toronto, Canada; 2) University of British Columbia, Vancouver, Canada.

Tay-Sachs and Sandhoff diseases are lysosomal storage disorders resulting from a deficiency in beta_hexosaminidase A (Hex-A). The chronic form of these disorders have about 5 percent of normal Hex-A activity and protein. However, asymptomatic individuals have been documented with only 10 percent residual Hex-A, implying a critical threshold of 5 to 10 percent of normal activity necessary to prevent storage.

We have showed in vitro that NAG-Thiazoline (NGT) acts as a chemical chaperone in fibroblast cell lines from chronic Tay-Sachs patient. It is believed to stabilize the mutant protein by transiently binding with its active site. Hence, this new drug is very promising as a potential treatment for adult Tay-Sachs and Sandhoff patients.

We have evaluated the acute toxicity of NGT at several doses, various time points and different route of administration in adult mice. The animals did not display any major behavioral modification or obvious clinical symptom. Different organs collected did not show any cytological and structural modification. Plasma and brain lysate showed an increase in Hex-A activity as compared to the control mice.

The increase in Hex-A activity observed in addition to the absence of toxicity provides hope for a possible treatment of chronic Tay-Sachs and Sandhoff diseases.

Searching for the stumbling block: possible mechanisms of skeletal muscle resistance to therapy in Pompe disease. *N. Raben, T. Fukuda, A.L. Gilbert, D. de Jong, E. Van Dyke, E. Ralston, P.H. Plotz.* NIAMS, NIH, Bethesda, MD.

Pompe disease, a deficiency of acid alpha-glucosidase (GAA), is a fatal lysosomal glycogen storage disorder affecting cardiac and skeletal muscle. Extensive recent studies have led to clinical trials with recombinant enzyme (rhGAA) for replacement therapy (ERT). The therapy held out the hope that the disease would henceforth be considered treatable. However, glycogen storage in skeletal muscle proves to be unexpectedly refractory to therapy. Attempts at gene therapy in our KO model by several groups, and our preclinical studies including escalating doses of the rhGAA, hepatic secretion of the transgenic enzyme, and transgenic GAA expression in muscle of the KO mice have all failed to completely remove the accumulated glycogen in skeletal muscle, indicating that the problem lies within the muscle cell itself. The skeletal muscle abnormalities go beyond the lysosome. Vacuoles of the endocytic pathway (the route of the rhGAA) - early and late endosomes, as well as the vacuoles of the autophagic pathway, which converges with the endocytic pathway - are all dramatically expanded in the diseased cells. Also, microarray analysis of skeletal muscle showed an upregulation of genes involved in glycolysis and glycogen metabolism, suggesting an increased energy demand. Not all muscle cells respond equally to ERT, however. Type I fibers clear glycogen well in the KO mice, thus narrowing the problem to the type II fibers, mainly type IIb. Analysis of single muscle fibers from ERT-treated KO mice identified a subset of huge cell-interior rhGAA-negative lysosomes in type II fibers. These fibers have much lower levels of key proteins involved in endocytosis and trafficking of lysosomal enzymes, CI-MPR, clathrin, and AP-2, and they show large autophagosomes and autolysosomes containing mitochondria and cellular debris. Microarray data confirmed an upregulation of the genes involved in autophagy: beclin, Rab24, and LC3. The data suggest that inefficient entry of the rhGAA into the cells may be further blocked by increased autophagy in these therapy-resistant fibers. The findings may have important implications for designing a second generation of therapeutic drugs.

A clinical study of miglustat (Zavesca) in adult and juvenile patients with Niemann-Pick Type C disease: preliminary data. A. Rolfs, T. Böttcher. Dept Neurology, Univ Rostock, Rostock MV, Germany.

Purpose: Niemann-Pick Type C (NPC) is a rare neurovisceral disorder caused by a block in the translocation of LDL-derived cholesterol from lysosomes to the endoplasmic reticulum and the Golgi apparatus. Miglustat (Zavesca) is an orally administered small-molecule glucosyltransferase inhibitor which received EU and US approval for the treatment of Gaucher disease. Studies in animal models of Sandhoff and NPC diseases demonstrated a significant amelioration of disorder-dependent and progressive neuronal pathology. We designed a prospective, open label and non-comparative clinical study to examine the influence of Zavesca on neuropsychological symptoms in adult and juvenile NPC disease.

Methods: 12 consecutive patients over 12 years of age with genetically proven NPC disease will be enrolled and will receive 3x100 mg Zavesca every day for 18 months. Efficacy evaluations will include detailed neuropsychological measurements as well as SPECT and MRI of the brain. Safety evaluations will include vital sign measurements, physical examinations, adverse event assessment and neuropsychological as well as laboratory tests.

Results: To date, 3 patients (male 43y, male 17y, female 17y) are enrolled in the study and have been placed on medication for 7-11 months. Leading clinical feature in all cases are an ataxic-dystonic syndrome and intellectual decline over the past years. MRI evaluation revealed no symptoms of progressing leukencephalopathy. None had symptoms of polyneuropathy at entry nor developed specific signs as evidenced by clinical examination and senso-motor nerve conduction studies. Diarrhoea occurred infrequently in two patients after 2-3 weeks of administration but could easily be treated with loperamide. Neuropsychological studies, performed in two patients, revealed no ongoing intellectual decline over 9-11 months.

Conclusions: Preliminary results on 3 patients treated with Zavesca for 7-11 months, indicate that the treatment is well tolerated in patients with NPC, with no development of a polyneuropathy. The adverse event profile does not differ from that known from Gaucher patients. Additional intellectual impairment could not be observed.

Neu4, a novel human lysosomal lumen sialidase confers normal phenotype to sialidosis and galactosialidosis cells.

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Sialidosis and galactosialidosis are neurodegenerative lysosomal storage disorders characterized by deficiency of the lysosomal sialidase (Neu1) due to the mutations in the NEU1 gene (sialidosis) or in the gene encoding for protective protein/cathepsin A (galactosialidosis). The loss of Neu1 activity in both sialidosis and galactosialidosis patients results in abnormal accumulation of sialylated oligosaccharides and glycoproteins. For both diseases, early onset forms with severe CNS pathology and systemic organ involvement, as well as relatively mild late onset variants have been identified. No effective treatment for sialidosis and galactosialidosis patients has been developed so far. We describe a novel lysosomal lumen sialidase Neu4 encoded by NEU4 gene on human chromosome 2. We showed that Neu4 has enzymatic activity towards the artificial sialidase substrate, 4-Mu-NANA as well as towards sialic acid-containing oligosaccharides, glycoproteins and glyco- sphingolipids. In contrast to Neu1, Neu4 is targeted to lysosomes by the mannose-6-phosphate receptor and does not require association with other proteins for enzymatic activity. Northern blot analysis demonstrated that Neu4 is expressed in all tissues studied with the highest level in skeletal muscle, heart, placenta and liver. Overexpression of recombinant Neu4 corrected extensive vacuolization due to the accumulation of lysosomal storage materials in Neu1-deficient cultured skin fibroblasts of sialidosis and galactosialidosis patients raising the possibility that the upregulation of Neu4 gene can be a promising approach for the treatment of sialidosis and galactosialidosis.

A hammerhead ribozyme prevents dominant-negative interference in gene therapy for phenylketonuria. C.

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Phenylketonuria (PKU), one of the most common human recessive diseases, is generally caused by defects in the enzyme phenylalanine hydroxylase (PAH). A mouse model for PKU, BTBR *Pah*^{enu2}, has a missense mutation (F263S) rendering PAH inactive. The mouse has classic PKU with elevated blood Phe levels, cognitive deficiencies, and maternal PKU syndrome. We corrected serum Phe levels in *Pah*^{enu2} mice using recombinant AAV2 vectors (rAAV2) containing the mouse PAH gene (mPAH). Although successful, unusually high vector doses were needed to achieve normal serum Phe levels.

Pah^{enu2} mice have one third the normal amount of PAH protein in liver. We hypothesized that, since the enzyme is a tetramer, endogenous mutant subunits could assemble with the gene therapy-introduced protein in a dominant-negative fashion reducing the total potential effectiveness of the treatment. To confirm this hypothesis, we designed a ribozyme against the mouse PAH mRNA and a ribozyme-resistant form of mPAH (mPAH-Hd). In transient cell transfections, a 1:1 molar ratio of mPAH-Hd to mPAH-F263S vector reduces PAH activity to one half that observed with mPAH-Hd alone. When the ribozyme is also expressed in the transfection, PAH activity is restored to near normal levels. Since the ribozyme cleaves F263S mRNA but does not cleave mPAH-Hd mRNA, we conclude that mixed tetramers of mPAH and mPAH-F263S proteins are causing the observed reductions in PAH activity.

We delivered an ineffective dose of mPAH-Hd rAAV2 vector along with increasing amounts of the ribozyme rAAV2 vector in male mice. At a 1:6 ratio of mPAH-Hd: ribozyme vector, we observed a decrease of 0.4mM in serum Phe levels for up to 15 weeks. While the decrease is significant when compared to pre-gene therapy levels, serum Phe has not reached a therapeutic range. Since separate vectors were used to deliver the ribozyme and PAH gene, simultaneous transduction of the same cell may not have occurred efficiently. A single combined ribozyme-mPAH-Hd cDNA rAAV vector has been constructed and animal experiments will soon be underway.

A five-year study of Aldurazyme for treatment of MPS I. *G. Eames¹, S. Swiedler², G. Cox³, L. Waber⁴, M. Lipson⁵, D. McMahon⁶, V. Proud⁷, R. Loge⁸, K. Dveirin⁹, E. Kakkis².* 1) Hematology/Oncology BMT Unit, Cook Children's Medical Center, Fort Worth, TX; 2) BioMarin Pharmaceutical, Novato, CA; 3) Genzyme, Cambridge, MA; 4) Univ. of Texas Southwestern Med. Ctr., Dallas, TX; 5) Kaiser Permanente, Sacramento, CA; 6) Carolinas Med. Ctr., Charlotte, NC; 7) Children's Hospital of the King's Daughters, Norfolk, VA; 8) Pioneer Medical Specialists, Dillon, MT; 9) Arizona Community Physicians, Tucson, AZ.

Objective: To demonstrate safety and efficacy of Aldurazyme (laronidase) in the reduction of lysosomal storage in Mucopolysaccharidosis I (MPS I). **Methods:** A Phase 1/2 open-label, multicenter study in 10 patients. Patients received 100 U/kg (0.58 mg/kg) laronidase weekly for a total of 269 to 288 weeks. The primary endpoint for the 5-year extension study was 50% reduction of uGAG (urinary GAG) levels. **Results:** Mean age was 12.3 years (range 5-22) and 80% had Hurler-Scheie syndrome. Three patients expired during the 5-year study at weeks 103, 137 and 234; none of the deaths was related to laronidase. Seven patients completed over 5 years on study and 6 of the 7 patients were compliant with weekly infusions. Mean reduction in uGAG excretion from pretreatment to end of study for the 6 compliant patients was 73.3%. Four of these 6 patients had uGAG levels approaching normal range for age. The seventh patient had a 68.5% reduction in uGAG levels at 3 years, but became non-compliant during the next 2 years and the uGAG returned to baseline levels. Data show that uGAG levels increase by 6% if 1 infusion is missed, and increase by 12% if 2 consecutive infusions are missed. The most frequently reported AEs occurred during infusion, i.e., urticaria, headache, edema, pruritus. All patients developed antibodies to laronidase though there was no apparent relationship between antibody levels and changes in urinary GAG excretion or infusion related hypersensitivity-type reactions. **Conclusion:** Compliance with weekly infusions of laronidase results in a consistent reduction of uGAG levels and an acceptable long-term safety profile.

Using clinical genetics to identify validated drug targets and a shorter route to drug development. *Y.P. Goldberg, J. MacFarlane, B. Popovich, R. Fraser, R. Kamboj, S.N. Pimstone, R. Sherrington, M.R. Hayden.* Xenon Pharmaceuticals Inc., Vancouver, BC, Canada.

We have developed an innovative clinical genetics approach to identify crucial disease-causing genes which serve as targets for novel drug development. Our approach examines Mendelian families having the opposite protective phenotype to the disease we intend to treat. Since most human genetic disease mutations are loss-of-function, therapeutic antagonists to the target will have the desired and clinically relevant outcome in humans, resulting in an easier and more efficacious drug development pathway. To identify targets for the treatment of osteoporosis, we studied the opposite phenotype disorder, sclerosteosis, characterized by increased osteoblastic activity resulting in desirable symmetrical increased bone density. The causal gene, sclerostin, is a primary transcriptional repressor of osteoblast activity. Thus sclerostin antagonists, currently in drug development, will produce a phenotype reminiscent of sclerosteosis, thereby improving bone density in patients with osteoporosis. To address the important unmet medical need of the Metabolic syndrome, we studied the Asebia mouse with low body weight, low plasma triglycerides and enhanced insulin sensitivity. The Asebia mouse was found to have a mutation in Stearoyl-CoA desaturase 1 (SCD1), a key enzyme in fat metabolism. Thus inhibition of SCD1 in humans will reproduce the desirable phenotype with beneficial impact on metabolic and body weight parameters. We have developed small molecule inhibitors to this target for the treatment of Metabolic syndrome. Anemia of Inflammation (AI), characterized by low serum iron concentration is another important area of unmet medical need. To develop therapeutic targets for AI, we identified opposite phenotype families with the severe iron overload recessive disorder, Juvenile hemochromatosis. We identified the causal gene, hemojuvelin, which is now entering drug development for treatment of the iron disturbances of AI. By focusing on the clinical phenotype, we have identified novel drug targets to address diseases with unmet medical needs.

Clinical Response and Molecular Evidence in Patients with Machado-Joseph Disease under Treatment of Lamotrigine. C. Kuo¹, C. Liu^{1,2,3}, H. Hsu⁴, B. Wang⁷, M. Hsieh^{5,6}. 1) Vascular and Genomic Research Center, Changhua Christian Hospital, Changhua, Taiwan; 2) Department of Neurology, Chung Shan Medical University and Chung Shan Medical University Hospital, Taiwan; 3) Department of Neurology, Changhua Christian Hospital, Taiwan; 4) Institute of Medicine, Chung Shan Medical University, Taiwan; 5) Department of Biology, Tunghai University, Taiwan; 6) Life Science Research Center, Tunghai University, Taiwan; 7) Ctr Medical Genetics, Changhua Christian Hospital, Taiwan.

Machado-Joseph disease (MJD) is an autosomal dominant spinocerebellar degeneration characterized by a wide range of clinical manifestations, including ataxia, progressive external ophthalmoplegia, pyramidal and extra pyramidal signs, dystonia with rigidity and distal muscular atrophies. Unstable CAG trinucleotide repeat expansion in MJD gene on the long arm of chromosome 14 was identified as the pathologic mutation of MJD. We present our study of the clinical response of lamotrigine (LTG) on six patients in their early ataxia stage of MJD. LTG was found to effectively relieve gait disturbance in these six MJD patients based on test scoring in the improvement of single leg standing and tandem gait index. Furthermore, the expression of ataxin-3 in MJD lymphoblastoid cells with and without LTG treatment was assessed by Western blot analysis. While leaving the normal ataxin-3 intact, extracellular application of LTG (50 to 300M) decreased the expression of mutant ataxin-3 in a dose-related manner. Decreased expression of mutant ataxin-3 seemed to be one of the possibilities to explain the effect of LTG on MJD patients with early stage of gait disturbance.

Pharmacological correction of VLCAD deficiency by fibrates. *F. Djouadi¹, F. Aubey¹, A.W. Strauss², J. Bastin¹.* 1) INSERM U393, Hosp Necker, Paris, France; 2) Vanderbilt University, Nashville, Tennessee, TN.

VLCAD (Very Long Chain AcylCoA Dehydrogenase) catalyses the first step of mitochondrial fatty acid oxidation (FAO). VLCAD deficiency has variable clinical presentations, from a severe childhood form with cardiomyopathy and high mortality to milder adult form with isolated skeletal muscle involvement. The severity of this disease is correlated to the nature of mutation and to the level of residual FAO. We therefore tested a possible stimulation of FAO by fibrates in primary culture of fibroblasts from patients diagnosed with various forms of VLCAD deficiency. Cell exposure to 500M bezafibrate for 48 h resulted in a marked increase in 3H-Oleate (+59 to +284%) and 3H-palmitate (+199 to +509%) oxidation rates in cell lines from 4 different patients presenting with mild VLCAD deficiency. FAO levels were similar in treated deficient or control fibroblasts, demonstrating complete restoration of FAO flux in VLCAD deficient cells treated by bezafibrate. Mutation analysis showed that this correction could be achieved in cells carrying the G520A or T848C VLCAD missense mutation. Bezafibrate effects were related to increases in VLCAD mRNA (x1.7) and protein (x2.6) levels, as shown by quantitative RT-PCR and western-blot studies, and this is consistent with a drug-induced stimulation of VLCAD gene transcription via PPAR (peroxisome proliferator activated receptor). We also used this cell system to test a possible response to PPAR agonists of other genes responsible for FAO defects. In preliminary experiments, bezafibrate was found to increase mRNA levels of CPT1-1A (liver form of Carnitine Palmitoyl Transferase; +126%), ETFDH (Electron Transfert Factor Dehydrogenase; +69%) and MCAD (Medium Chain AcylCoA Dehydrogenase; +58%) in control cells. Further studies will allow us to determine if defects in these other FAO enzymes can also be corrected in deficient patient cells. In conclusion, our studies of VLCAD-deficient cells further support the notion that pharmacological stimulation of PPAR signaling pathway provides a way to correct some inborn errors of metabolism in patient cells, as our group initially established for the CPT2 deficit.

Sodium Phenylbutyrate Increases Full-length SMN2 mRNA in SMA Lymphocytes. *M. Ganta, S.M. Grzeschik, C.H. Wang.* Neurology, Stanford University, Stanford, CA.

Spinal muscular atrophy (SMA) is an autosomal recessive disorder characterized by the degeneration of the spinal cord motor neurons and muscular atrophy. Over 95% of SMA patients lack the functional SMN1 gene but all patients retain at least one copy of the highly homologous SMN2 gene. Unlike SMN1, SMN2 produces very little full-length (FL) mRNA but generates mostly truncated (TR) mRNA lacking exon 7. Increasing the level of full-length SMN transcripts produced by SMN2 is a potential therapeutic strategy for SMA. We tested the ability of sodium phenylbutyrate (SPB) to increase production of full-length SMN mRNA in cultured lymphoblastoid cell lines. 18 cell lines derived from 14 SMA patients (4 type I, 5 type II, 5 type III) and 4 control individuals were treated with SPB in six concentrations and over three time points along with appropriate vehicle-treated controls. RT-PCR and quantitative densitometry were used to detect the changes of the SMN mRNA species. Western blot analysis was used to detect changes in SMN protein levels. SPB treatment resulted in a significant dose-dependent and time-related increase in the SMN FL/TR transcript ratio. Peak response occurred at a concentration of 500g/ml and 48h time point. At 5000g/ml dose RNA and protein levels were not detectable indicating toxicity to the cells. The FL/TR transcript ratios at 0, 0.5, 5, 50, and 500g/ml SPB concentrations, after 48h of treatment, were: type I - 0.76, 0.69, 0.74, 0.77, 1.43*; type II - 0.66, 0.71, 0.74, 0.78, 1.78***; type III - 0.63, 0.63, 0.80, 0.88, 2.15*. Using a concentration of 500g/ml, the ratios after 24, 48 and 72h of SPB treatments were: type I - 1.23, 1.54*, 1.60; type II - 1.80, 1.85*, 1.63*; type III - 1.70*, 1.18* , 1.20. Non-SMA cell lines did not show significant changes at any concentration or time point. SMN protein levels increased in 2 of 6 (33%) SMA cell lines tested and one of 2 control cell lines after SPB treatment. These results indicate that SPB increases the full-length mRNA production from the SMN2 gene in human SMA lymphoblastoid cell lines and can be considered as a potential therapeutic agent for SMA. *P< 0.05, **P< 0.01, ***P< 0.001.

Chemical chaperones protect from effects of apoptosis-inducing signal sequence mutation in carbonic anhydrase IV recently identified in retinitis pigmentosa (RP17). *W.S. Sly, G. Bonapace, A. Waheed, G.N. Shah.* Dept Biochem & Molec Biol, St Louis Univ Sch of Med, St Louis, MO.

Carbonic anhydrase IV (CA IV) is a GPI-anchored enzyme highly expressed in the choriocapillaris of the human eye. We and our collaborators from South Africa recently identified an R14W mutation in the signal sequence of CA IV in patients with the RP17 form of autosomal dominant retinitis pigmentosa, and showed it induced apoptosis in a large fraction of transfected COS cells expressing the mutant, but not the wild-type, CA IV (Rebello et al. Proc Natl Acad Sci USA 101:6617-6622, 2004). Here we report that chemical chaperones can largely block the apoptotic-inducing effects of this mutation in transfected COS cells and suggest they could be therapeutic in this protein folding disease.

Transfected COS cells expressing the wild-type CA IV or R14W CA IV with or without co-transfecting plasmids expressing other secretory proteins were treated with chemical chaperones and the enzyme activities were measured. Cells were also subjected to pulse-chase experiments followed by Western blot analysis. Immunohistochemical staining was used to quantitate cells positive for CA IV, BiP, PERK, CHOP (markers of the UPR and ER stress) and TUNEL reaction.

These experiments showed that several well-characterized CA inhibitors largely prevented the adverse effects of expressing R14W CA IV in transfected COS cells. Specifically, they prevented the accelerated turnover of the mutant protein, the upregulation of BiP, PERK, and CHOP, the inhibition of production of other secretory proteins expressed from co-transfecting plasmids, and the induction of apoptosis, all characteristics of transfected cells expressing R14W CA IV. Furthermore, treatment of transfected COS cells with 4-phenylbutyric acid, a non-specific chemical chaperone used in other protein folding disorders, dramatically reduced the apoptosis-inducing effect of expressing R14W CA IV cDNA in transfected COS cells. These experiments suggest a promising approach to treatment of RP17 that might delay the onset or possibly prevent this autosomal dominant form of retinitis pigmentosa.

Molecular mechanisms by which the G269S Hexosaminidase A mutant prevalent in patients with late-onset Tay-Sachs disease is stabilized by inhibitors acting as pharmacological chaperones. *M. Tropak¹, J. Blanchard³, S. Reid², A. Leung¹, J. Callahan¹, S. Withers², E. Brown^{3,4}, D. Mahuran¹.* 1) Metabolism, Hospital For Sick Children, Toronto, Ontario, CANADA; 2) Biochemistry, University of British Columbia, Vancouver, British Columbia, CANADA; 3) McMaster HTS lab, McMaster University, Hamilton, Ontario, CANADA; 4) Biochemistry, McMaster University, Hamilton, Ontario, CANADA.

In Tay-Sachs and Sandhoff diseases, inadequate levels of Hexosaminidase A (- dimer) in the lysosome results in the intralysosomal accumulation of GM2 ganglioside. We have previously shown that Hexosaminidase activity can be enhanced in fibroblast cell lines from patients bearing the Gly269Ser mutation in the alpha subunit or the Pro508Ser mutation in the beta-subunit, respectively, by treating the cells with Hexosaminidase inhibitors acting as pharmacological chaperones. To address the mechanism by which enzyme activity is enhanced, we have used differential centrifugation analysis to demonstrate that dissociation of wild type Hexosaminidase B is decreased in the presence of the inhibitor N-acetyl glucosamine thiazoline. Furthermore, we show that the G269S Hexosaminidase A mutant in comparison to wild type enzyme dissociates more readily at 42C. When the mutant enzyme is incubated in the presence of Hex inhibitors dissociation is attenuated at 42C. These results are consistent with the model that inhibitors of Hexosaminidase facilitate dimerization of the mutant alpha/beta- subunits enabling exit of the enzyme from the endoplasmic reticulum and transport to the lysosome. We have applied these approaches to characterize a panel of novel Hexosaminidase inhibitors identified in the Maybridge library of compounds by High Throughput Screening. These compounds represent potential novel pharmacological chaperones for the treatment of the chronic forms of Tay-Sachs and Sandhoff diseases.

Pulmonary function findings in patients with infantile Pompe disease. *P.S. Kishmani¹, J. Mackey¹, S. DeArme¹, J. Harris¹, J.T. Koepke¹, M.T. McDonald¹, R.M. Kravitz².* 1) Dept Ped Medical Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Pediatric Pulmonary Diseases, Duke University Medical Center, Durham, NC.

Background: Pompe disease (PD) is a lysosomal storage disorder caused by deficiency of acid -glucosidase. It presents early in life with hypotonia, generalized muscle weakness, and hypertrophic cardiomyopathy. This form is fatal; most die by 1 year due to cardiac and/or respiratory failure. Supportive care may transiently prolong lifespan, but is not associated with long term survival. Previous trials of recombinant human acid -glucosidase (rhGAA) enzyme replacement therapy (ERT) have shown encouraging results. Given potential treatment, optimal respiratory management is essential to improve outcome. Assessment of pulmonary function (PF) is not routinely performed. To date, no study has systematically examined PF in PD.

Design: 8 patients (2 requiring tracheostomies and mechanical ventilation) were enrolled in 2 open label studies of rhGAA. PF [crying vital capacity (CVC), oxygen saturation (SaO₂), ventilation (EtCO₂), and Negative Inspiratory Flow Manuever (NIFM)] were obtained on enrollment and after 3 and 6 months of ERT.

Results: Enrollment SaO₂ was normal in 5/8 and EtCO₂ was normal in 8/8 patients. CVC and NIFM were decreased in all 8 patients. After 3 months of ERT, data was available for 7/8 patients. 3/5 of unventilated and 1/2 of ventilated patients remained on room air. 5/5 of unventilated and 1/2 of ventilated patients had normal EtCO₂. 6/7 patients had improved CVCs and 4/5 had improved NIFMs. After 6 months of ERT, data was available for 6/8 patients. 3/4 of unventilated and 2/2 of ventilated patients remained on room air. EtCO₂ remained normal in all 6 patients. 5/6 patients had improved CVCs and 5/6 had improved NIFMs when compared to enrollment.

Conclusions: PF abnormalities were seen in all patients. CVC and NIFM were the most affected but improved during ERT. SaO₂ also improved. EtCO₂ remained normal. The expected worsening of PF seen in historic norms did not occur. ERT appears to improve PF in patients with PD.

Sleep study findings in patients with infantile Pompe disease. *R.M. Kravitz¹, J. Mackey², S. DeArme², J. Harris², J.T. Koepke², M.T. McDonald², P.S. Kishnani².* 1) Pediatric Pulmonary Diseases, Duke University Medical Center, Durham, NC; 2) Pediatric Medical Genetics, Duke University Medical Center, Durham, NC.

Background: Pompe disease (PD) is a lysosomal storage disorder caused by deficiency of acid -glucosidase. It presents early in life with hypotonia, generalized muscle weakness, and hypertrophic cardiomyopathy. This form is fatal; most patients die by 1 year due to cardiac and/or respiratory failure. While supportive care may transiently prolong lifespan, it is not associated with long term survival. Previous trials using recombinant human acid -glucosidase (rhGAA) enzyme replacement therapy (ERT) have shown encouraging results. Given potential treatment, optimal respiratory management is essential to improve outcome. Assessment of nocturnal ventilation is not routinely performed. To date, no study has systematically examined sleep disordered breathing (SDB) in PD.

Design: 6 patients were enrolled in 2 open label studies of rhGAA. Sleep studies were obtained upon enrollment and after 6 months of therapy.

Results: 3/6 patients had no evidence of SDB or hypoventilation. 1/6 had SDB but no hypoventilation. 2/6 had both SDB and hypoventilation. 0/6 of these patients had histories that would have suggested the presence of SDB. The 2 patients with hypoventilation were treated with BiPAP therapy which was tolerated well. To date, 4/6 have had repeat sleep studies performed (3 after 6 months of therapy; 1 after 3 months). Of the 3 patients with initially normal studies, 1 remained normal; 1 developed mild SDB with no hypoventilation; the third developed hypoventilation without SDB. This patient was started on BiPAP, which was well tolerated. The fourth follow up study (done on a patient previously started on BiPAP) showed no SDB or hypoventilation.

Conclusions: SDB is a complication of PD which presents before the patient is symptomatic. It is responsive to BiPAP therapy. No patient required tracheostomy and mechanical ventilation, which would have been expected based on historic norms. ERT appears to be effective at preventing the expected development of respiratory failure in PD.

Blockade of aconitase activity in Friedreichs ataxia : Potential consequences for cellular fate? *A. Derre, N. Khadom, A. Munnich, P. Rustin.* Inserm U 393, Paris, France.

Friedreichs ataxia is the most common recessive ataxia which is frequently associated with life-threatening cardiomyopathy, and is usually due to a GAA triplet expansion in the first intron of the frataxin gene. In human, the resulting frataxin loss of function in the mitochondrial matrix specifically hampers the synthesis of iron-sulfur clusters (ISC) for ISC-containing proteins needed in different cell compartments. Among these proteins, both cytosolic and mitochondrial aconitases are the most severely affected. The consequences of aconitase impairment are presumably multiple, including general disturbance of iron metabolism through the conversion of cytosolic aconitase to its iron-responsive protein form which controls cell iron-homeostasis, through metabolic disturbance with blockade of the Krebs cycle in the mitochondria and of isocitrate production in the cytosol. The consequences of isocitrate shortage are not known and we investigated these by inhibiting aconitase activity in control cultured fibroblasts using the specific aconitase inhibitor, fluorocitrate. At the used concentrations (16 and 40 M), fluorocitrate causes citrate accumulation and subsequent cell death. Attempts were made to protect cells against fluorocitrate-induced aconitase blockade by providing a series of compounds which may counteract putative consequences of isocitrate shortage, especially the NADPH generation necessary for maintenance of reduced glutathione pool. None of the tested compounds were successful in preventing cell death, suggesting an alternative mechanism for the cell death observed. Interestingly, providing iron, in either oxidized or reduced form, with isocitrate afforded limited protection against fluorocitrate-induced cell death. Our study establishes the importance of i) the aconitase catalytic activity, and ii) the balance of iron/organic acid, for cultured skin fibroblast survival (and presumably in other tissues as well).